PLANT AVAILABLE PHOSPHORUS FROM BONE CHAR AND BIOCHAR ADDITIONS IN A PHOSPHORUS-FIXING SOIL

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Marie Jasmijn Zwetsloot

August 2013

ABSTRACT

Managing phosphorus (P) plant availability in soils and efficient usage of P fertilizers are essential for agricultural sustainability worldwide. Since P is an immobile nutrient, preventing P-deficiency in crops becomes especially challenging in highly weathered, acid soils that are prone to strong P adsorption. With the rapid depletion of global P reserves, finding alternatives to rock phosphate-dependent fertilizers and management practices that enhance P availability in agricultural ecosystems will become even more critical. Recycling P from slaughterhouse waste into bone char fertilizers through pyrolysis could make human P usage more sustainable and affordable. Animal bone is rich in calcium phosphates (CaP), but many of its uses are banned due to the risk of Bovine Spongiform Encephalopathy (BSE). Charring bone could overcome this problem through heat sterilization.

Bone char has been used as P fertilizer for centuries; however, little is known about how pyrolysis production conditions influence the chemical P characteristics of bone fertilizers. Applying organic matter in form of biomass or biochar as strategy to enhance P availability and reduce P adsorption to mineral oxides also warrants further investigation. Moreover, few studies address soil management and plant foraging strategies to improve P accumulation simultaneously and little is known about how plants with different rooting strategies obtain access to P sources with varying solubility.

The study discussed in the first chapter of this thesis used X-ray Absorption Near-Edge Structure (XANES) spectroscopy and wet-chemistry extractions to determine (1) how pyrolysis temperature and biomass additions influence P chemistry in bone char and (2) how CaP crystallinity affects the fertilizer potential of bone char. The second chapter discusses an abiotic incubation and pot trial with maize (*Zea mays* L., variety B73) designed with the objectives (1)

to test the P fertilizer characteristics of bone char in comparison to TSP fertilizer in a P-fixing soil, (2) to determine the effect of co-pyrolyzed biochar on P availability, and (3) to analyze how maize roots with varying soil exploration capacity access different P sources. Olsen-extractions and anion-exchange resins (AER) were used to determine P availability in incubated soils. Maize mutants without root hairs and arbuscular mycorrhizae (AM, *Glomus clarum*, strain WV235) inoculants were used to alter the soil exploration capacity of maize. Morphological characteristics of roots were quantified after scanning using the software package WinRHIZO Pro 2007d.

Results show that increasing pyrolysis temperature enhances CaP crystal formation. Co-pyrolysis with biomass reduces CaP crystallization. Higher CaP crystallinity is associated with lower water-soluble P and higher formic acid-extractable P. Charring bone at 350°C results in a 67% increase in resin-P when incubated in a P-fixing soil. The addition of wood biochar decreases resin-P by 14-26%, while uncharred wood increases resin-P by 23%. This may indicate that pyrolysis reduces the potential of wood biomass to enhance P availability. The 13-19% increase in Olsen-P from co-pyrolyzed rendered bone and wood biochar is explained by a decrease in CaP crystallinity by wood biomass during pyrolysis. When associated with AM, maize had similar access to P from rendered bone char and TSP fertilizer. Maize receiving co-pyrolyzed rendered bone-wood char application demonstrated lower plant P accumulation, suggesting that wood biochar may have had a negative effect on AM colonization. Future research should concentrate on optimizing the effects of biochar on P availability from bone char during pyrolysis and in soils. In addition, P availability from bone char in different soil environments should be tested.

BIOGRAPHICAL SKETCH

Marie Jasmijn Zwetsloot grew up in Westernieland and Eenrum near the Wadden Sea in the north of the Netherlands. In 2005, she received a scholarship to complete her last two years of high school at the United World College of the Atlantic in St. Donat's in Wales. She graduated with a Bachelor of Arts degree focusing on international sustainable development from University College Maastricht (UCM) in January 2011. During her time at UCM, she was an AIESEC Dutch student ambassador in Peru, studied abroad at Universidad de San Francisco de Quito in Ecuador, worked at the Princeton University Center for Human Values, farmed at the Natick Community Organic Farm in the United States and conducted field research with cacao farmers in Nicaragua. Visiting smallholder farms in Latin America, hiking through the Andes and camping in tropical rainforest sparked her interest in soil-plant relations. The year before starting a research master's degree at Cornell University, she took classes in plant and soil science at Wageningen University.

ACKNOWLEDGEMENTS

I would like to thank my major advisor Dr. Johannes Lehmann and minor advisor Dr. Taryn Bauerle for their guidance and inspiration throughout this research. I am also very grateful for the technical training and help from Akio Enders and Kelly Hanley. Working together with other members of the Lehmann lab has been a great learning experience, and I would like to especially thank my fellow graduate students Rachel Hestrin, Dorisel Torres and Thea Whitman for discussing ideas and collaborative grant writing.

I have very much appreciated the support from members of the Crop and Soil Sciences Department and Horticulture Department. In particular, I would like to express many thanks to Bob Schindelbeck for helping me with a soil water retention analysis, the Buckley lab for letting me use their photospectrometer, and Dr. Neil Mattson for giving me access to his root scanner. Moreover, I would like to thank the project collaborators at Jimma University and CARE-International in Ethiopia for discussing research plans and helping me export 500 kg of soil to the USA.

Completing a research master's degree at Cornell University would not have been possible without the funding from Fulbright International and the Huygens Talent Scholarship Programme, for which I am extremely thankful. I also acknowledge the financial research support by the Bradfield Award, Towards Sustainability Foundation, the P3 grant by the Environmental Protection Agency (EPA), CARE-Cornell Impact through Innovations Fund (CARE-IIF) and McKnight Foundation.

TABLE OF CONTENTS

CHAPTER 1

RECYCLING SLAUGHTERHOUSE WASTE INTO FERTILIZER: HOW DO PYROLYSIS TEMPERATURE AND BIOMASS ADDITIONS AFFECT PHOSPHORUS CHEMISTRY IN BONE CHAR?[1]

Abstract

Rock phosphate mining for phosphorus (P) fertilizer has led to a rapid depletion of global P reserves and excessive use of P fertilizer has led to pollution of aquatic ecosystems. Recycling slaughterhouse waste into bone char fertilizers through pyrolysis - the heating of material under low oxygen conditions - has the potential to make human P usage more sustainable. The objectives of this study were (1) to identify the effect of pyrolysis temperature, rendering bone and mixing bone with a biomass source before pyrolysis on chemical P properties of bone char and (2) to analyze the relation between these production parameters and the P fertilizer potential of bone char. Pyrolysis feedstock sources included bone with meat residue, rendered bone, corn stover and hard wood. Bone char and bone-biomass chars were produced at 220, 350, 550 and 750°C. Results showed that higher pyrolysis temperatures increased calcium phosphate (CaP) crystallinity estimated by X-ray Absorption Near Edge Structure (XANES) spectroscopy. P fraction incorporated into crystalline hydroxyapatite (HA) structure increased from 0 to 69.3 % for bone and meat residue and from 19.7 to 94.9% for rendered bone meal. P resembling

[1] To be submitted to a journal under Zwetsloot, M. J.; Lehmann, J.; Solomon, D.

hydroxyapatite (HA) was 37% higher in rendered bone than bone with meat residue. The addition of biomass to bones before pyrolysis reduced the amount of P present in HA form by 83% for wood and 95% for corn. A high degree of CaP crystallinity was correlated with low water-extractable P, but high formic acid-extractable P, which reached values that were only 24% lower than those in Triple Superphosphate (TSP) fertilizer and two- to five-fold higher than in rock phosphate at similar solubility assessed by formic acid extraction

Introduction

Human intervention in the phosphorus (P) cycle has turned rock phosphate into a finite resource as it is more rapidly mined than deposited (Neset and Cordell 2012; Abelson 1999). Some predict a global phosphate crisis with P reserves running out worldwide within the next 50-100 years (Cordell et al. 2009; Gilbert 2009). Fertilizer production utilizes 80% of the rock phosphate that is mined annually (Smil 2000). The use of P fertilizer in modern agriculture has also led to excessive runoff into waterways with negative impact for aquatic ecosystems (Vaccari 2009; Tilman et al. 2001). Hence, making agricultural fertilizer production more sustainable is one of the first steps to a more efficient and less polluting P cycle.

One proposed solution is to recover P from organic waste disposal (Cordell et al. 2011) and convert it into fertilizer. This option includes producing bone char out of slaughterhouse waste by means of pyrolysis, the heating of materials under low oxygen conditions. The European Union has banned the use of bone and meat meal in cattle feed preparation due to the risk of spreading Bovine Spongiform Encephalopathy (BSE) (Cascarosa et al. 2012). Pyrolysis overcomes this problem through heat sterilization. Unlike rock phosphate, bone char does not

contain toxic metals such as nickel, chrome and cadmium (Deydier et al. 2005; Chaala and Roy 2003), making it a less harmful soil amendment.

Calcium phosphates are the primary constituents of bone. Besides the large mineral structure fraction (60%), bone consists of 40% enamel (Posner 1985). The bone mineral structure is commonly classified as biological apatite, yet the exact crystallography and formation chemistry of bone are still being investigated. In comparison to geological apatite such as hydroxyapatite (HA), biological apatite has a smaller crystal size, more carbonate substitutions and a significant OH deficiency, overall resulting in greater solubility (Wopenka and Pasteris 2005; Boskey 2007).

Pyrolysis modifies the chemical and physical structure of biological apatite. Production conditions including pyrolysis temperature significantly alter the yield and quality of bone char (Ayllon et al. 2006). In addition, the meat:bone ratio can drastically change the end product (Cascarosa et al. 2011). The thermal decomposition mainly occurs between 250°C and 500°C and ends after 750°C (Ayllon et al. 2006; Conesa et al. 2003). With increasing pyrolysis temperature, bone char shows higher calcium phosphate (CaP) crystallinity, smaller ash particle size, fewer carboxyl and amide functional groups, more macro-pores and higher metal concentration in comparison to the original feedstock (Novotny et al. 2012; Deydier et al. 2005; Chaala and Roy 2003).

However, the majority of studies about pyrolysis and (co-)gasification of slaughterhouse waste are primarily interested in how production conditions alter its potential as fuel source (Cascarosa et al. 2012) and optimize tar production (Fedorowicz et al. 2007). While there has been an attempt to measure characteristics of bone char, the solid product yield (Chaala and Roy 2003), the effect of production parameters on the chemical properties of bone char related to its

efficacy as agricultural fertilizer is largely unknown. Although studies have demonstrated the P fertilizer potential of bone char (Warren et al. 2009; Mondini et al. 2008), questions about how pyrolysis temperature and feedstock type change the characteristics of bone char important for the development of effective P fertilizers remain unanswered.

Increasing pyrolysis temperature is hypothesized to lead to higher CaP crystallinity, suggesting a decrease in solubility (Jaynes, Moore, and Miller 1999). Previous studies have added a carbon source such as coal to the pyrolysis or gasification process of meat and bone meal to prevent stickiness from fatty meat (Cascarosa et al. 2012). Co-pyrolyzing bones with a biomass source could also influence CaP crystal formation and may thereby offer opportunities to manipulate P solubility and availability of bone char fertilizers. A concurrent production of biochar from biomass could aid in making P better available to plants (Cui et al. 2011; Nelson et al. 2011), but no information about co-pyrolysis of biomass with bones has been published.

The objectives of this study are (1) to determine how pyrolysis temperature, rendering bone and mixing bone with a biomass source before pyrolysis affect CaP crystallinity; and (2) to determine the relation between these production conditions and the P fertilizer potential of bone char as represented by P solubility and plant availability indicators.

Materials and Methods

Material Preparation

Bones with meat residue were collected from Dudley Poultry in Middlesex, NY, USA. Rendered bone meal was purchased as organic fertilizer from The Espoma Company 1929 (Milville, NJ, USA). Hard wood chips (80% red maple, 20% sugar maple) were obtained from Robinson Lumber (Owega, NY, USA). Corn harvest residue consisting of stalk and stover came

from Cornell Farm Services (Ithaca, NY, USA). Bone with meat residue, wood and corn were oven-dried at 60°C for 5 days and milled to a particle size < 2 mm. An aliquot of 300 g of each material was individually pyrolyzed at a heating rate of 2.5°C min-1 and maintained at 220, 350, 550 and 750°C for 45 min in a 9.93 L stainless steel chamber fitted into a muffle furnace. The chamber was swept with argon gas and had rotating paddles for homogenization. In addition, bone with meat residue mixed with wood or corn and rendered bone mixed with wood at mass ratio of 1:1 were charred under the same pyrolysis conditions. Char yield was measured. Samples with pyrolysis temperature of 350°C and above were ground with mortar and pestle and sieved to < 74 µm particle size for X-ray Absorption Near Edge Structure (XANES) spectroscopy and to 74-150 µm particle size for chemical analyses. Bones and biomass samples at 60 and 220°C were ground by a Thomas Wiley Mill mesh size 60 for chemical analyses and additionally ground using a ball grinder for XANES spectroscopy.

Rock phosphate from Idaho, USA supplied by the Espoma Company 1929 (Milville, NJ, USA) and Greenkeeper's Secret Triple Super Phosphate (TSP, T&N, Incorporated, Foristell, MO, USA) were ground to 74-150 µm particle size for chemical analyses and used as comparison for the bone fertilizers.

X-ray Absorption Near Edge Structure (XANES) Spectroscopy

Phosphorus K-edge XANES spectroscopy characterization of the char samples was carried out at beam line X19-A of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory in Upton, NY, USA. Samples were mounted in the center of an acrylic sample holder (diameter = 1.3 cm, depth = 0.5 mm) and covered with a 5-µm thick polycarbonate (0.2 mil) Mylar X-ray film. A Passivated Implanted Planar Silicon (PIPS)-detector

measured the P K-edge fluorescence spectra of the samples in a helium-purged chamber. Reference P minerals and salts were obtained from a chemical supplier or synthesized as indicated: hydroxyapatite (HA, Sigma Aldrich), synthesized octacalcium phosphate (OCP) which was verified for its chemical characteristics using X-ray powder diffraction analysis, β-tri-calcium phosphate (TCP, Fluka), aluminum phosphate (AP, Fisher Scientific), iron(III)phosphate dihydrate (IPD, Sigma Aldrich), dibasic calcium phosphate (DCP, Sigma Aldrich), dicalcium phosphate dihydrate (DCPD, Riedel de Häen), calcium phosphate monobasic (CPM, Fisher Scientific), sodium phosphate dibasic monohydrate (SPDM, Mallinckrodt Baker Inc.), phytic acid sodium salt hydrate (PASH, Sigma Aldrich), 1-naphthyl phosphate (NP, Sigma Aldrich), diphenyl phosphate (DPP, Sigma Aldrich) and triphenylphosphine (TPP, Sigma Aldrich). X-ray energy was calibrated to the P K-edge using TCP as standard and placing its maximum absorption peak at 2149 eV as reference energy (E0). Single scans of all samples were collected at region boundaries -15, -5, 15, 30 and 60, and interval region steps 1, 0.1, 0.02 and 1. As the bone char samples all contained high P concentrations, it was not necessary to go further below or above the P K-edge for background subtraction.

Chemical Analyses

Char samples were analyzed for plant-available P with a 2% formic acid extraction as this method showed highest correlation with P plant uptake from high-ash biochars (Wang et al. 2012). Orthophosphate content of extracts was analyzed with the ascorbic acid method (Murphy and Riley 1962). Water-soluble P, calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), sodium (Na) and sulfur (S) were extracted by shaking char samples in deionized water at a 1:150 solid-solution ratio for 16 h and filtering with 2V Whatman qualitative filter paper (Hollister et

al. 2013). The modified dry-ashing method (Enders and Lehmann 2012) was used to obtain total P, Ca, Mg, K, Fe, Na and S. Elemental contents were quantified in the extracts by inductively coupled plasma atomic emission spectrometry (ICP-AES Thermo Jarrell Ash 166 Trace Analyzer, Thermo Jarrell Ash Corporation, Franklin, MA, USA). To determine pH, samples were shaken in water at 1: 20 solid-solution ratio for 90 minutes and analyzed for pH (Orion 3 Star, Thermo Scientific Inc., Beverly, MA, USA)

Data Analysis

The program ATHENA of the IFEFIT software package (Ravel and Newville 2005) was used for data processing and analysis of the XANES spectra. After checking the accuracy of the pre- and post-edge parameters of randomly selected scans, spectra were subjected to automatic background correction executed by ATHENA. Linear combination fitting was performed over the spectral region from 15 eV below to 30 eV above the P absorption edge. To allow for uncertainty, weights of the standards were not fixed to 1. All 13 standards were used in the analysis. A maximum of 4 standards were allowed to contribute to a fit. First, the groups of organic, inorganic and CaP standards were separately fitted. Standards that never obtained a weight other than 0 were removed from the analysis. The second round of linear combination fitting used the selected organic, inorganic and CaP standards simultaneously. Fits were compared according to their chi-square values. Fits of standards were qualitatively interpreted. OCP, a precursor of bone apatite, was used as indicator of low CaP bone crystallinity and HA as indicator of high CaP bone crystallinity. In addition, the ratio of HA/OCP provided a measure for the degree of bone crystallinity. Lastly, expected curves of the biomass-bone mixtures were calculated arithmetically based on the ratios of total phosphorus contents and spectra of the

individual materials. They were compared to the measured spectra by plotting them in the same graph.

Statistical analyses were performed with software package JMP Pro 10.

Results and Discussion

XANES Spectral Features of Standards

CaP standards were selected according to their solubility which is reported to decrease in the following order CPM > DCPD > DCP > TCP > OCP > HA (Montastruc et al. 2003; Jaynes et al. 1999). Except for TPP, the white line energy (absorption edge) fell approximately at 2149 eV (Figure 1.1) and an oxygen oscillation peak occurred at around 2166 eV. The CaP standards, other inorganic standards, and organic P standards were distinctively different from one another. The shoulder at around 2151 eV was a unique feature for all CaP standards. This secondary peak sharpened and the absorption edge peak widened for more crystalline CaP references such as HA and OCP, which indicates lower solubility and higher thermodynamic stability (Peak et al. 2002; Beauchemin et al. 2003). For HA, OCP and TCP, a tertiary sharp peak appeared at 2159 eV. CaP standards with a $CaHPO_4$ such as DCP, DCPD and CPM also have a peak but more rounded at the same energy range. This observation is in line with the P K-edge XANES spectra of Sato et al. (2005) and Ingall et al. (2011) but not with the data published by Peak et al. (2002) that do not show a tertiary peak at 2159 eV for DCP. This could be attributed to the difference between our sample synthesized by Sigma Aldrich and a natural DCP sample (monetite) used by Peak et al. (2002).

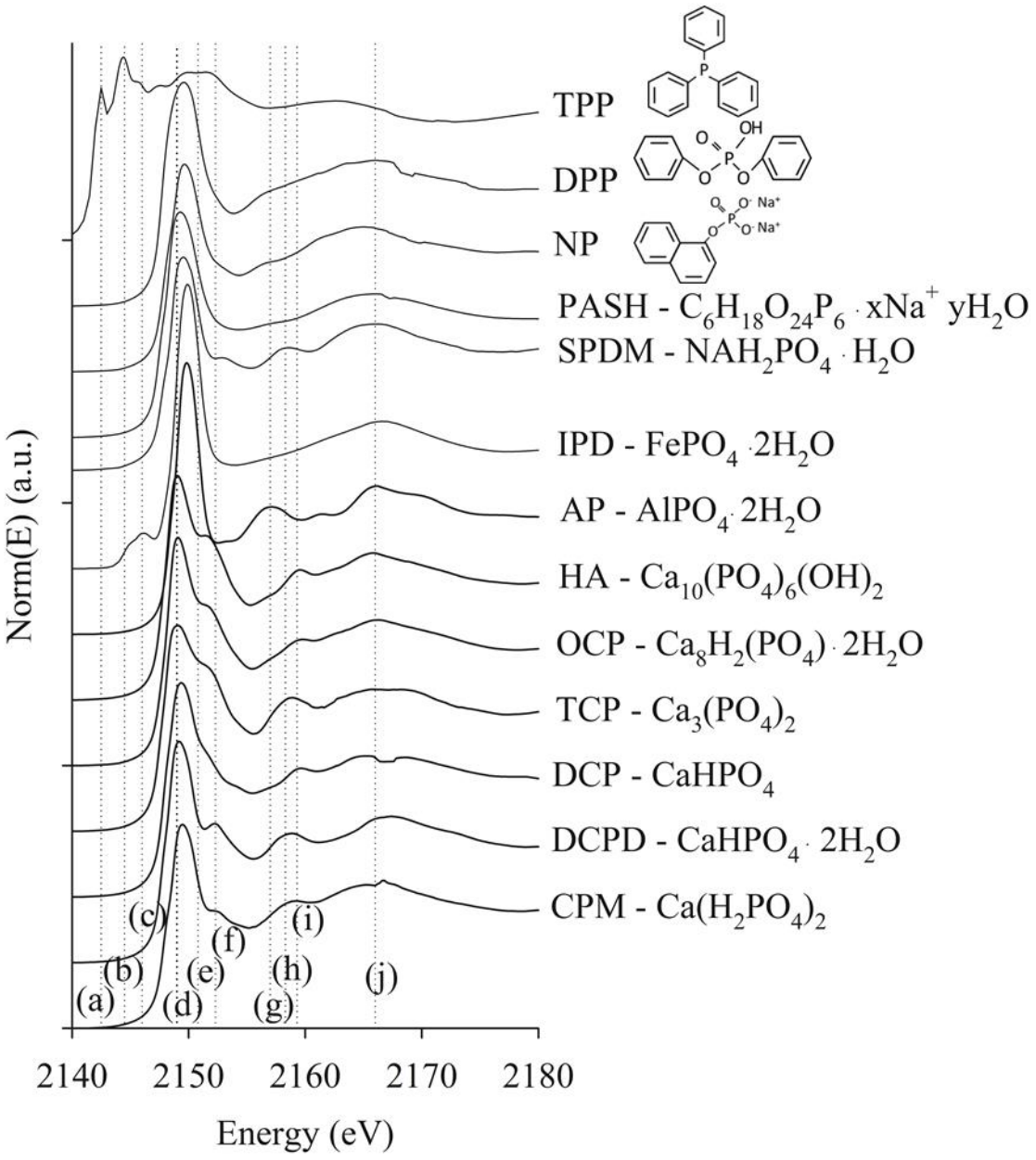

Figure 1.1. Phosphorus K-edge XANES spectra for P standards species. The dotted lines indicate energy levels that characterize unique spectral features for different P species: (a) triphenylphosphine (TPP), (b) triphenylphosphine (TPP), (c) iron(III)phosphate dehydrate (IPD), (d) absorption edge, (e) octacalcium phosphate (OCP) and hydroxyapatite (HA), (f) calcium phosphate (CaP) species, (g) aluminum phosphate (AP), (h) sodium phosphate dibasic monohydrate (SPDM), (i) β-tri-calcium phosphate (TCP), octacalcium phosphate (OCP) and hydroxyapatite (HA), (j) oxygen oscillation.

IPD was characterized by a pre-absorption edge peak at 2146 eV. AP had a secondary broad peak at 2157 eV. SPDM showed two small peaks in between the absorption edge and oxygen oscillation peak: one similar to the CaP shoulder of more soluble CaP compounds at around 2151 eV and one peak at 2158 eV. The AP spectrum was consistent with other studies, while the post-absorption edge spectrum of IPD was smoother than in other studies (Peak et al. 2002; Ingall et al. 2011; Sato et al. 2005; Franke and Hormes 1995).

The main absorption edge peaks of PASH, NP and DDP were shifted the most to the right by 0.2-0.5 eV. The same phenomenon occurred with organic standards reported by Brandes et al. (2007). While the shape and width of the absorption edge peak slightly differed among these three organic phosphate standards, they had no clear additional distinctive features besides the oxygen oscillation peak. The spectrum of PASH was similar to the scans of other phytic acid species from previous studies (Toor et al. 2005; Brandes et al. 2007). The white line energy peak of TPP occurred at 2144.5 eV, which is still in the pre-edge of all other reference compounds. TPP also showed a secondary peak before its major absorption peak at 2142.5 eV. There was no oxygen oscillation peak because TPP was the only compound with no oxygen bound to P. This standard was included to identify P atoms in heterocycles.

Phosphorus Chemical Structures in Bone Char Samples

In the case of rendered bone and bone with meat residue, increasing pyrolysis temperature led to widening of the absorption edge peak at 2149 eV, sharpening of the CaP shoulder at 2151 eV and the peak characteristic for apatite-like CaP minerals at 2159 eV (Figure 1.2), demonstrating an increase in CaP crystallinity. This is consistent with results from X-ray diffractrometry (Deydier et al. 2005). At lower temperatures, the spectra of the OCP standard

resembled those of rendered bone and bone with meat residue spectra better than HA as shown by Figure 1.2 and the linear combination fitting results (Table 1.1). However, with an increase in temperature bone with meat residue moves from 0% to a 69.3 % match with HA and rendered bone from 19.7% to a 94.9 % match with HA. It is important to note that the linear combination fitting results of OCP and HA should not be interpreted literally. HA used in this study was synthesized and thus showed a higher degree of CaP crystallinity than biological apatite. OCP, the acidic precursor of HA in bone (Bodier-Houlle et al. 1998), has a similar chemical CaP structure as HA but also contains water in its crystal lattices (Johnsson and Nancollas 1992). Bone is a disordered and less crystalline apatite in comparison with synthetic and geological apatite and could show more resemblance to synthesized OCP. Therefore, we will interpret a high OCP fit as bone apatite with low crystallinity and a high HA fit as bone apatite with high crystallinity.

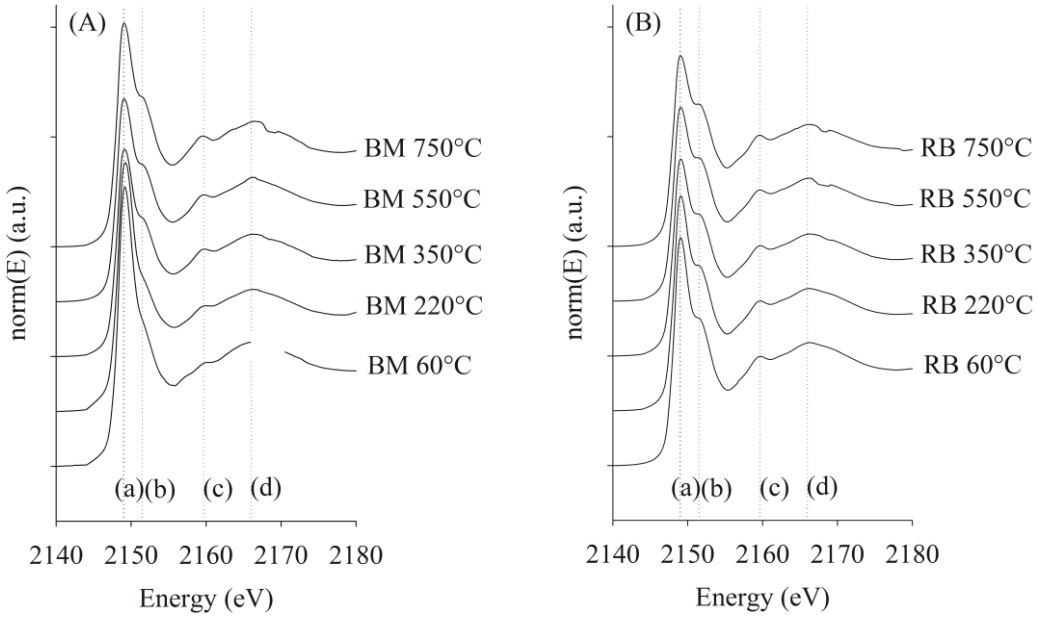

Figure 1.2. Phosphorus K-edge XANES spectra for bone with meat residue (BM) and rendered bone (RB) at different pyrolysis temperatures. The dotted lines indicate energy levels that characterize unique spectral features for different P species: (a) absorption edge, (b) octacalcium phosphate (OCP) and hydroxyapatite (HA), (c) OCP and HA, (d) oxygen oscillation.

Table 1.1. Linear combination fitting results of different feedstock mixtures dried or pyrolyzed at different temperatures. Feedstock includes bone with meat residue (BM), rendered bone (RB), bone with meat residue and wood (BW), bone with meat residue and corn (BC), rendered bone and wood (RBW). P species are octacalcium phosphate (OCP), hydroxyapatite (HA), iron(III)phosphate dehydrate (IPD), phytic acid sodium salt hydrate (PASH), diphenyl phosphate (DPP).

Feedstock	**T (°C)**	**OCP (%)**	**HA (%)**	**IDP (%)**	**PASH (%)**	**DPP (%)**	χ^2
BM	60	71.9		15.9	22.6		7.09
BM	220	59.2		11.3	36.4		3.13
BM	350	63.1	27.9	2.8	9		0.38
BM	550	38.1	53.7		9.7		0.30
BM	750	33.2	69.3	3.7			1.26
RB	60	83.4	19.7	2.1			1.27
RB	220	61.7	39.1	2.6			0.54
RB	350	17.3	81.1	3.1			0.07
RB	550	4.2	93.3	4.2			0.07
RB	750	6.2	94.9				0.05
BW	220	67.3		12.1	8.2	23.1	5.13
BW	350	90.7		1.3		12.7	1.14
BW	550	96.9		0.4		6.2	0.71
BW	750	89.2	11.6	1.9			0.36
BC	220	60.7		11.2	37.6		4.41
BC	350	83.2		7.6	14.6		2.46
BC	550	99.1		5.3			0.88
BC	750	100	3.8	4.3			2.01
RBW	220	88.8	11.3	2.5			0.59
RBW	350	64.1	36.5	1.5			0.44
RBW	550	26.9	72.3	3			0.26
RBW	750	33.2	68.5				0.16

The rendered bone spectra showed a better fit with HA than bone with meat residue even at lower temperatures (Table 1.1). Rendering removes fatty meat residue, leaving a purer source of CaP mineral showing a higher resemblance with HA even without charring. Moreover, less contamination of fatty meat could facilitate CaP crystal formation at a faster rate during pyrolysis.

Biomass additions in the form of wood and corn reduced CaP crystal formation during pyrolysis. The chars made from bone with meat residue and wood, bone with meat residue and corn and rendered bone-wood have higher fit values for OCP than for HA at all temperatures in comparison to the chars made from rendered bone and bone with meat residue (Table 1.1). Moreover, the expected arithmetic mean based on total P concentration of the source of bone and biomass at a specific temperature was not equal to the obtained spectrum of the XANES scan (Figure 1.3 and Figure 1.4), especially for chars made from bone and meat residue mixed with wood or corn biomass. There are several mechanisms that could explain the inhibition of CaP crystal formation. First, the addition of corn or wood biomass resulted in an increase of carbon and organic acids. Adsorption and chemical bonding of these molecules onto CaP crystal surfaces could have blocked sites acting as nuclei for continued crystal growth (Alvarez et al. 2004; Grossl and Inskeep 1991, 1992). Second, metal ions from the biomass sources could have decreased crystal formation through impeding the growth of nuclei or poisoning the substrate resulting in heterogeneous nucleation (Madsen 2008). Strong metal inhibitors are Cu^{2+}, Zn^{2+} (Madsen 2008) and Mg^{2+} (Martin and Brown 1997), none of which were present in high amounts. Cu^{2+}, Zn^{2+} were below the detection limit of the ICP-AES and higher Mg concentration did not correlate with reduced crystal growth (supporting information Figure-1.S2 and 1.S4).

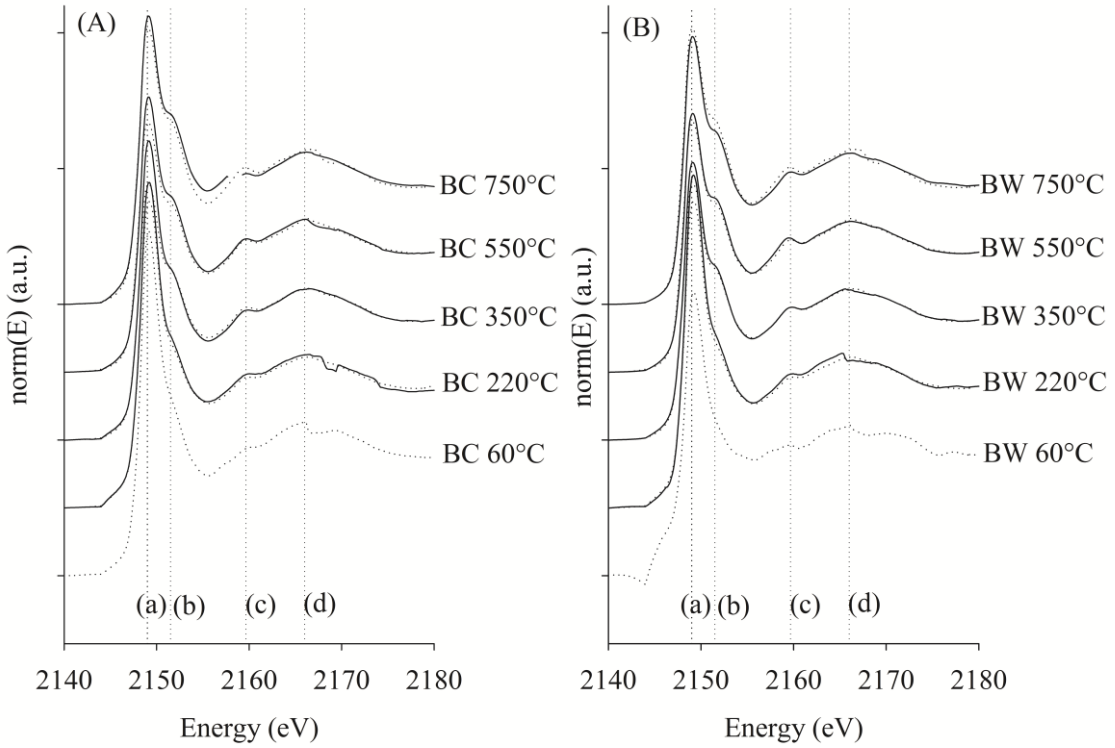

Figure 1.3. Phosphorus K-edge XANES spectra for mixtures of bone and biomass (solid line) with expected arithmetic sum of individual spectra (dotted line): (A) bone with meat residue and corn biomass (BC) and (B) bone with meat residue and wood biomass (BW). The vertical dotted lines indicate energy levels that characterize unique spectral features for different P species: (a) absorption edge, (b) octacalcium phosphate (OCP) and hydroxyapatite (HA), (c) OCP and HA, (d) oxygen oscillation.

The organic P standards PASH and DPP constituted a small part of the spectra from bone with meat residue alone and mixed with wood or corn biomass, but not of the spectra from rendered bones and only at low pyrolysis temperatures (Table 1.1). This indicates that rendering bone removes most organic P being part of the fatty meat residue and that with increasing pyrolysis temperature organic P seems to be broken down and transformed into inorganic P. NP and TPP did not show fits with any of the samples and DPP as part of the feedstock (Table 1.1). These results suggest that no heterocyclic P forms were generated through pyrolysis.

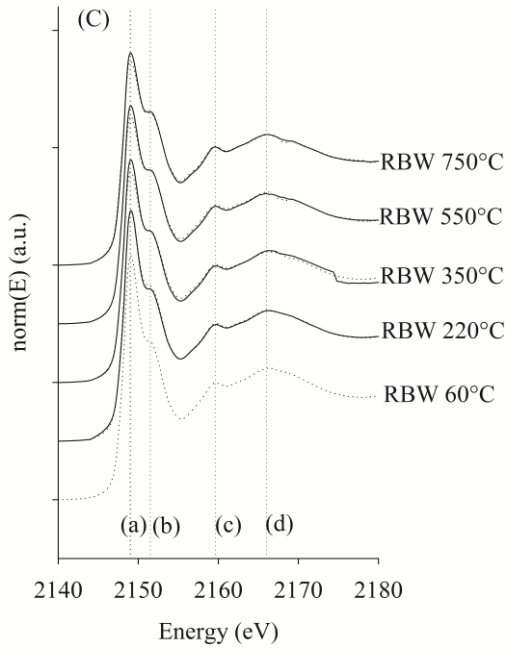

Figure 1.4. Phosphorus K-edge XANES spectra for mixtures of rendered bone and wood biomass (solid line) with expected arithmetic sum of individual spectra (dotted line). The vertical dotted lines indicate energy levels that characterize unique spectral features for different P species: (a) absorption edge, (b) octacalcium phosphate (OCP) and hydroxyapatite (HA), (c) OCP and HA, (d) oxygen oscillation.

Phosphorus Concentration, Solubility and Availability in Samples

All samples were enriched in total P with increasing pyrolysis temperature (Table 1.2). P vaporization and particulate transfer to air is generally low in comparison to N, S, C and K (Raison et al. 1985; DeLuca et al. 2009; Knudsen et al. 2004; Huang et al. 2011), resulting in a greater P concentration in high temperature bone chars. Ca was enriched to a higher degree than P. This increased the molar Ca/P ratio at high pyrolysis temperatures (Table1. 2). A higher Ca/P ratio is an indicator of increased CaP crystallinity. This is in line with our XANES results: above a Ca/P ratio of 1.6 the HA/OCP ratio goes up suggesting increased crystallinity (Figure 1.5A).

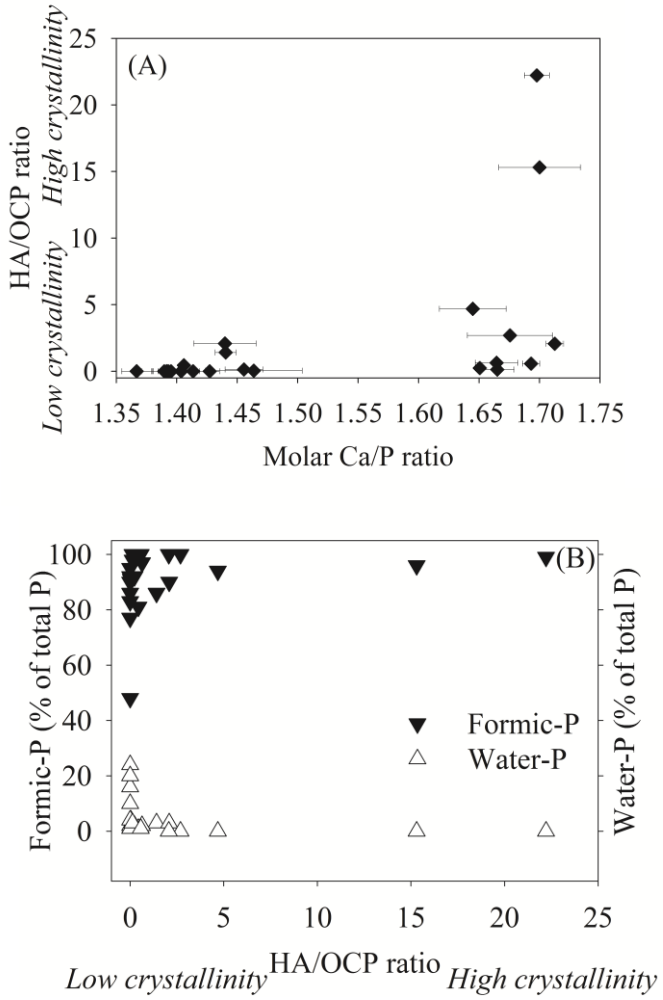

Figure 1.5. Calcium phosphate crystallinity relationships: (A) relation between two indicators of CaP crystallinity molar calcium/phosphorus (Ca/P) ratio and hydroxyapatite/octacalcium phosphate (HA/OCP ratio), (B) effect of HA/OCP ratio as indicator of CaP crystallinity on water-soluble and formic acid extractable P

Table 1.2. Chemical Characteristics of Sample pH, total P, total Ca, formic-P and water-P (means and standard deviation; n=3). Feedstock include bone with meat residue (BM), rendered bone (RB), bone with meat residue and wood biomass (BW), bone with meat residue and corn biomass (BC) and rendered bone and wood biomass (RBW). * Nutrient characteristics of these samples were calculated as an arithmetic mean from the two materials it was composed of and pH content was not measured.

Feedstock	T (°C)	pH	total-Ca (mg Ca g^{-1})	total-P (mg P g^{-1})	formic-P (% of total P)	water-P (% of total P)	mass yield (% of initial)
BM	60	6.3 ± 0.01	58.7 ± 6.9	32.6 ± 3.6	86.1 ± 9.6	15.6 ± 1.5	100
BM	220	6.7 ± 0.01	57.3 ± 3.3	31.3 ± 1.6	48.0 ± 10.0	10.2 ± 0.6	90
BM	350	8.4 ± 0.82	151.7 ± 6.5	83.4 ± 3.6	81.0 ± 3.6	1.2 ± 0.1	38
BM	550	10.6 ± 0.01	194.1 ± 3.2	104.1 ± 1.4	86.2 ± 0.9	2.9 ± 0.1	25
BM	750	11.0 ± 0.01	204.1 ± 5.7	109.5 ± 0.3	89.5 ± 1.8	3.0 ± 0.1	26
RB	60	7.3 ± 0.01	183.4 ± 10.5	85.9 ± 4.7	92.2 ± 2.9	2.0 ± 0.2	100
RB	220	6.8 ± 0.01	189.4 ± 6.0	87.9 ± 0.4	96.7 ± 4.5	2.0 ± 0.1	-
RB	350	7.5 ± 0.00	270.6 ± 7.2	127.1 ± 1.3	94.1 ± 5.5	0.3 ± 0.0	68
RB	550	9.2 ± 0.06	307.2 ± 5.4	139.9 ± 4.4	98.8 ± 1.0	0.5 ± 0.0	60
RB	750	10.2 ± 0.06	337.1 ± 8.2	153.2 ± 2.2	95.9 ± 3.0	0.3 ± 0.0	54
BW*	60	n/a	29.9 ± 3.4	16.3 ± 1.8	86.1 ± 9.6	15.6 ± 1.5	100
BW	220	6.5 ± 0.01	27.8 ±1.5	15.4 ± 0.9	95.1 ± 7.9	20.3 ± 1.0	94
BW	350	8.3 ± 0.08	72.1 ± 2.7	39.7 ± 1.0	77.0 ± 5.3	1.5 ± 0.1	39
BW	550	10.1 ± 0.06	95.4 ± 3.7	53.1 ± 2.5	86.1 ± 5.4	3.7 ± 0.3	30
BW	750	10.5 ± 0.01	106.3 ± 5.1	56.4 ± 1.5	97.7 ± 6.3	2.7 ± 0.2	28
BC*	60	n/a	30.4 ± 3.4	16.6 ± 1.8	85.5 ± 9.5	16.3 ± 1.5	100
BC	220	6.7 ± 0.02	25.4 ± 1.5	14.3 ± 0.6	92.0 ± 8.8	24.0 ± 1.0	101
BC	350	9.6 ± 0.08	69.8 ± 2.4	37.8 ± 1.6	82.9 ± 3.0	1.8 ± 0.1	6
BC	550	10.3 ± 0.01	100.6 ± 2.0	55.7 ± 1.7	90.1 ± 0.6	4.4 ± 0.2	23
BC	750	10.4 ± 0.00	110.1 ± 2.7	58.1 ± 1.0	91.3 ± 4.7	4.3 ± 0.2	17
RBW	60	n/a	92.3 ± 5.3	43.0 ± 2.3	92.2 ± 2.9	2.0 ± 0.2	100
RBW	220	6.6 ± 0.01	102.1 ± 5.3	47.4 ± 1.6	101.0 ± 1.3	3.4 ± 0.1	91
RBW	350	7.2 ± 0.06	224.4 ± 4.2	102.4 ± 1.7	99.7 ± 3.3	0.6 ± 0.1	51
RBW	550	8.8 ± 0.04	218.7 ± 3.7	100.9 ± 3.8	101.0 ± 2.9	0.5 ± 0.0	44
RBW	750	10.1 ± 0.01	258.3 ± 4.5	116.6 ± 2.6	102.1 ± 4.4	0.4 ± 0.0	41

Formic-P (in % of total P) also increased with temperature (Table 1.2), suggesting that bone chars produced at higher pyrolysis temperature provided larger amounts of plant available

P. However, the opposite was true for water-P (in % of total P), which decreased with an increase in production temperature (Table 1.2). Even at lower temperatures, formic-P was substantially higher than water-P (Table 1.2). This latter trend has been observed in other studies; while the water-soluble P fraction might be an underestimate of the amount of P available for plants, the acid-soluble (formic) P fraction of the bone char is an overestimate of immediate available P (Ylivainio et al. 2008). Overall, amounts of available P in bone char reached 14.7±0.5 %. This P concentration is five times higher than in Idaho rock phosphate two and a half times as high as GAFSA rock phosphate (Warren et al. 2009) and only 24% lower than the formic acid extractable P in TSP fertilizer (supporting information Table 1.S1 and Table 1.S2). Increased CaP crystallinity as represented by the HA/OCP ratio had a significant negative correlation with water-P and a significant positive correlation with formic-P (Table 1.3), resulting in lower water-P and higher formic-P (Figure 1.5B). Previous studies confirm that more crystalline CaP such as HA are more stable and, therefore, have lower solubility in water than OCP (Lindsay and Moreno 1960; Moreno et al. 1960; Moreno et al. 1968). On the other hand, HA has a higher pH and might be more acid-soluble than other CaP structures. This can explain the increase in formic-P with temperature. As wood and corn additions led to lower crystallinity, water-P increased and formic-P decreased.

Table 1.3. Spearman's correlations with hydroxyapatite/octacalcium phosphate (HA/OCP ratio) as indicator of calcium phosphate (CaP) crystallinity and formic acid-extractable P (formic-P), water-soluble P (water-P) and pH.

Variable	By variable	Spearman's ρ	Prob > |ρ|
HA/OCP	Formic-P	0.55	0.008
HA/OCP	Water-P	-0.77	< 0.0001
HA/OCP	pH	0.48	0.024
HA/OCP	Temperature	0.45	0.037

Char from rendered bone meal contained higher total P and formic-P, but had lower water-P than that from bones with meat residue (Table 1.2). Rendering bones with meat residue made the end product a purer source of mineral P and removed the more soluble elements from the fatty meat. Higher initial concentrations of Ca and P could also have facilitated crystal formation.

In summary, P K-edge XANES analysis and chemical P extractions showed that pyrolysis temperature increased CaP crystallinity, total P content and formic-P, but decreased P solubility in water. Rendering slaughterhouse waste before pyrolysis resulted in a purer mineral P source with higher total P contents and higher CaP crystallinity. Mixing wood or corn biomass with bone largely reduced CaP crystal formation during pyrolysis. Hence, all three variables – pyrolysis temperature, rendering and biomass addition – can be used to manipulate P characteristics of a bone-based fertilizer that are desirable for a particular farming system. Future research should test a variety of bone chars in diverse cropping systems and soil types to determine which bone fertilizers perform best in different settings.

Supporting Information

Supporting data associated with this article follows.

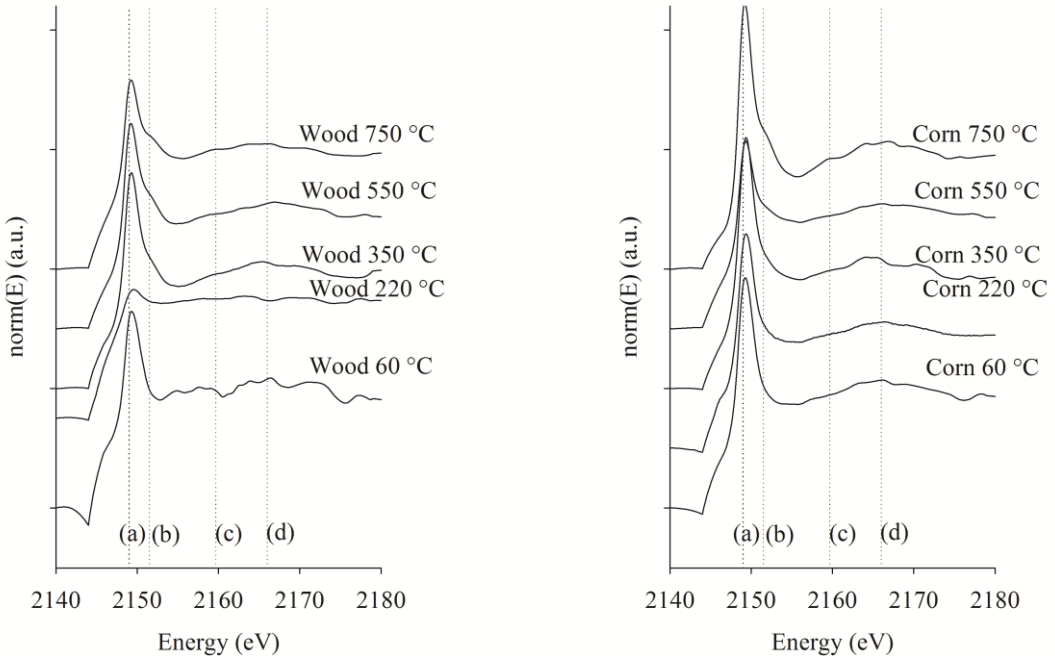

Figure 1.S1. Phosphorus K-edge X-ray Absorption Near Edge Structure spectroscopy of wood and corn biomass at different pyrolysis temperatures. The dotted lines indicate energy levels that characterize unique spectral features for different P species: (a) absorption edge, (b) CaP shoulder, (c) secondary peak of octacalcium phosphate (OCP) and hydroxyapatite (HA), (d) oxygen oscillation.

Table 1.S1. Chemical characteristics of commercial fertilizers Idaho Rock Phosphate and Triple Super Phosphate (TSP) fertilizer: total calcium content (total-Ca), total phosphorus content (total-P), formic acid-extractable phosphorus (formic-P) and water-extractable phosphorus (water-P) (means and standard deviations, n =3).

	total-Ca (mg Ca g^{-1})	**total-P (mg P g^{-1})**	**formic-P (% of total P)**	**water-P (% of total P)**
Rock phosphate	224.0 ± 4.2	84.8 ± 1.4	27.9 ± 0.6	0.4 ± 0.1
TSP	161.0 ± 5.2	205.3 ± 1.8	94.5 ± 2.0	100.0

Table 1.S2. Formic acid extractable P (means and standard deviations, n = 3) of bone fertilizers, rock phosphate from Idaho (Espoma Company 1912) and Triple Super Phosphate fertilizer (TSP). Bone fertilizer feedstock includes bone with meat residue (BM), rendered bone (RB), bone with meat residue and wood biomass (BW), bone with meat residue and corn biomass (BC) and rendered bone and wood biomass (RBW). *Not measured, but calculated as arithmetic mean of its two feedstock sources.

Feedstock	Temperature (°C)	Formic-P (mg P g^{-1} fertilizer)
BM	60	28.1±3.1
BM	220	15.0±3.1
BM	350	67.5±3.0
BM	550	89.8±0.9
BM	750	98.1±1.9
RB	60	79.1±2.5
RB	220	85.0±4.0
RB	350	119.6±7.0
RB	550	138.2±1.4
RB	750	147.0±4.7
BW*	60	14.1±1.6
BW	220	14.7±1.2
BW	350	30.6±2.1
BW	550	45.7±2.9
BW	750	55.1±3.5
BC*	60	14.2±1.6
BC	220	13.2±1.3
BC	350	31.3±1.1
BC	550	50.2±0.3
BC	750	53.1±2.7
RBW*	60	39.6±1.2
RBW	220	47.8±0.6
RBW	350	102.1±3.4
RBW	550	101.9±2.9
RBW	750	119.0±5.1
Rock phosphate	n/a	23.7±0.5
TSP fertilizer	n/a	194.0±4.1

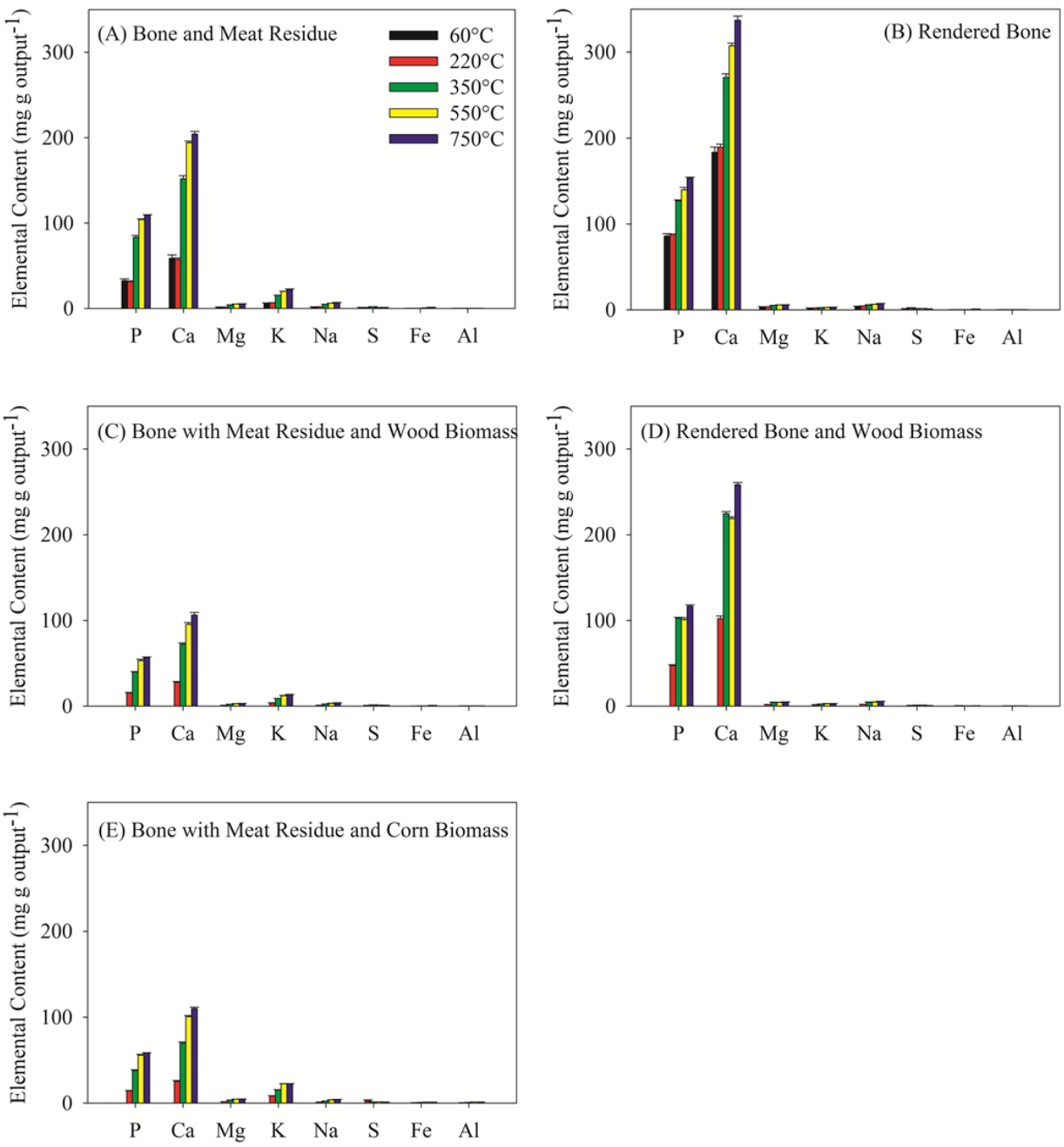

Figure 1.S2. Total elemental analysis of bone-based fertilizers at 60, 220, 350, 550 and 750°C. Elements include phosphorus (P), calcium (Ca), Magnesium (Mg), potassium (K) and sodium (Na).

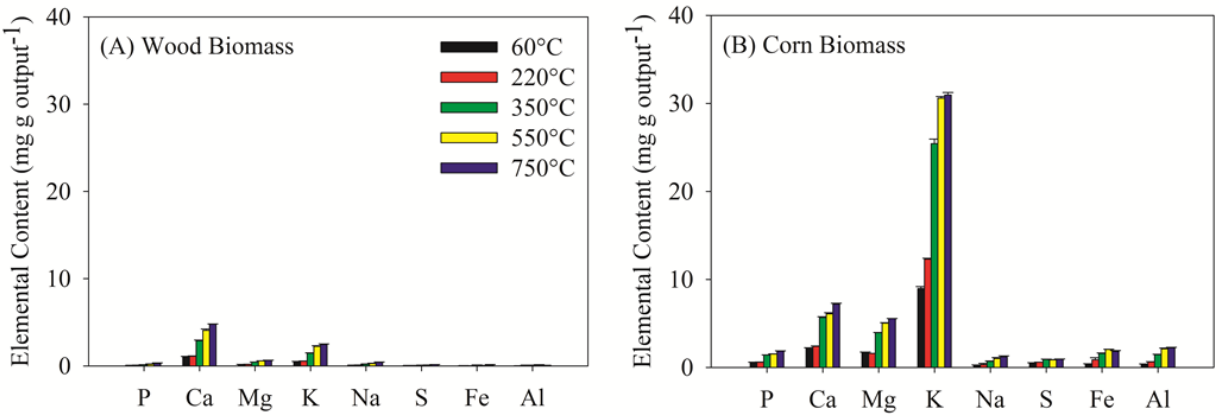

Figure 1.S3. Total elemental analysis of (A) wood and (B) corn biomass at 60, 220, 350, 550 and 750°C. Elements include phosphorus (P), calcium (Ca), Magnesium (Mg), potassium (K) and sodium (Na).

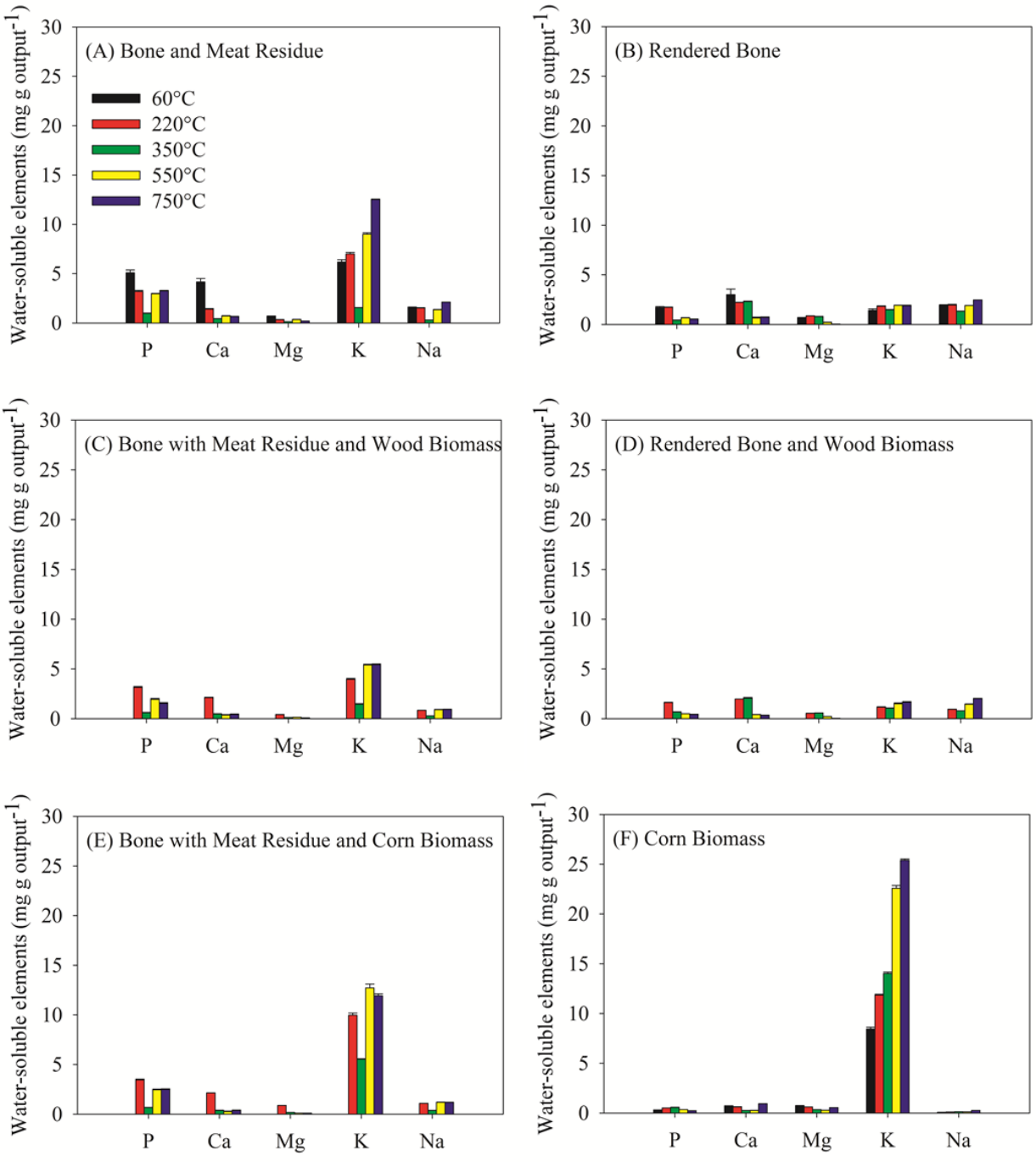

Figure 1.S4. Water-soluble elements of original feedstock and char at 60, 220, 350, 550 and 750°C: Elements include phosphorus (P), calcium (Ca), Magnesium (Mg), potassium (K) and sodium (Na).

References

Abelson, P. H. 1999. A potential phosphate crisis. *Science* 283 (5410).

Alvarez, R., L. A. Evans, P. J. Milham, and M. A. Wilson. 2004. Effects of humic material on the precipitation of calcium phosphate. *Geoderma* 118 (3-4).

Ayllon, M., M. Aznar, J. L. Sanchez, G. Gea, and J. Arauzo. 2006. Influence of temperature and heating rate on the fixed bed pyrolysis of meat and bone meal. *Chemical Engineering Journal* 121 (2-3).

Beauchemin, S., D. Hesterberg, J. Chou, M. Beauchemin, R. R. Simard, and D. E. Sayers. 2003. Speciation of phosphorus in phosphorus-enriched agricultural soils using X-ray absorption near-edge structure spectroscopy and chemical fractionation. *Journal of Environmental Quality* 32 (5).

Bodier-Houlle, P., P. Steuer, J. C. Voegel, and F. J. G. Cuisinier. 1998. First experimental evidence for human dentine crystal formation involving conversion of octacalcium phosphate to hydroxyapatite. *Acta Crystallographica Section D-Biological Crystallography* 54.

Boskey, A. L. 2007. Mineralization of bones and teeth. *Elements* 3 (6).

Brandes, J. A., E. Ingall, and D. Paterson. 2007. Characterization of minerals and organic phosphorus species in marine sediments using soft X-ray fluorescence spectromicroscopy. *Marine Chemistry* 103 (3-4).

Cascarosa, E., L. Gasco, G. Garcia, G. Gea, and J. Arauzo. 2012. Meat and bone meal and coal co-gasification: environmental advantages. *Resources, Conservation and Recycling* 59 (1).

Cascarosa, E., J. Becker, L. Ferrante, C. Briens, F. Berruti, and J. Arauzo. 2011. Pyrolysis of meat-meal and bone-meal blends in a mechanically fluidized reactor. *Journal of Analytical and Applied Pyrolysis* 91 (2).

Cascarosa, E., G. Gea, and J. Arauzo. 2012. Thermochemical processing of meat and bone meal: A review. *Renewable & Sustainable Energy Reviews* 16 (1).

Chaala, A., and C. Roy. 2003. Recycling of meat and bone meal animal feed by vacuum pyrolysis. *Environmental Science & Technology* 37 (19).

Conesa, J. A., A. Fullana, and R. Font. 2003. Thermal decomposition of meat and bone meal. *Journal of Analytical and Applied Pyrolysis* 70 (2).

Cordell, D., J. O. Drangert, and S. White. 2009. The story of phosphorus: Global food security and food for thought. *Global Environmental Change-Human and Policy Dimensions* 19 (2).

Cordell, D., A. Rosemarin, J. J. Schroder, and A. L. Smit. 2011. Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere* 84 (6).

Cui, H. J., M. K. Wang, M. L. Fu, and E. Ci. 2011. Enhancing phosphorus availability in phosphorus-fertilized zones by reducing phosphate adsorbed on ferrihydrite using rice straw-derived biochar. *Journal of Soils and Sediments* 11 (7).

DeLuca, T H, M D MacKanzie, and M J Gundale. 2009. Bio-char effects on soil nutrient transformation. In *Biochar for Environmental Management: Science and Technology*, edited by J. Lehmann, and S. Joseph. London: Earthscan Publications Ltd.

Deydier, E., R. Guilet, S. Sarda, and P. Sharrock. 2005. Physical and chemical characterisation of crude meat and bone meal combustion residue: "waste or raw material?". *Journal of Hazardous Materials* 121 (1-3).

Enders, A., and J. Lehmann. 2012. Comparison of Wet-Digestion and Dry-Ashing Methods for Total Elemental Analysis of Biochar. *Communications in Soil Science and Plant Analysis* 43 (7).

Fedorowicz, E. M., S. F. Miller, and B. G. Miller. 2007. Biomass gasification as a means of carcass and specified risk materials disposal and energy production in the beef rendering and meatpacking industries. *Energy & Fuels* 21 (6).

Franke, R., and J. Hormes. 1995. The P K-near edge absorption spectra of phosphates. *Physica B* 216 (1-2).

Gilbert, N. 2009. Environment: The disappearing nutrient. *Nature* 461 (7265).

Grossl, P. R., and W. P. Inskeep. 1991. Precipiation of dicalcium phosphate dihydrate in the presence of organic acids. *Soil Science Society of America Journal* 55 (3).

Grossl, P. R. 1992. Kinetics of octacalcium phosphate crystal growth in the presence of organic acids. *Geochimica Et Cosmochimica Acta* 56 (5):1955-1961.

Hollister, C. C., J. J. Bisogni, and J. Lehmann. 2013. Ammonium, Nitrate, and Phosphate Sorption to and Solute Leaching from Biochars Prepared from Corn Stover (Zea mays L.) and Oak Wood (Quercus spp.). *J. Environ. Qual.* 42 (1).

Huang, Y., H. Dong, B. Shang, H. Xin, and Z. Zhu. 2011. Characterization of animal manure and cornstalk ashes as affected by incineration temperature. *Applied Energy* 88 (3).

Ingall, E. D., J. A. Brandes, J. M. Diaz, et al. 2011. Phosphorus K-edge XANES spectroscopy of mineral standards. *Journal of Synchrotron Radiation* 18.

Jaynes, W. F., P. A. Moore, and D. M. Miller. 1999. Solubility and ion activity products of calcium phosphate minerals. *Journal of Environmental Quality* 28 (2).

Johnsson, M. S. A., and G. H. Nancollas. 1992. The role of brushite and octacalcium phosphate in apatite formation. *Critical Reviews in Oral Biology & Medicine* 3 (1-2).

Knudsen, J. N., P. A. Jensen, and K. Dam-Johansen. 2004. Transformation and release to the gas phase of Cl, K, and S during combustion of annual biomass. *Energy & Fuels* 18 (5).

Lindsay, W. L., and E. C. Moreno. 1960. Phosphate phase equilibria in soils. *Soil Science Society of America Journal* 24 (3).

Madsen, H. E. L. 2008. Influence of foreign metal ions on crystal growth and morphology of brushite (CaHPO4, 2H(2)O) and its transformation to octacalcium phosphate and apatite. *Journal of Crystal Growth* 310 (10).

Martin, R. I., and P. W. Brown. 1997. The effects of magnesium on hydroxyapatite formation in vitro from CaHPO4 and Ca-4(PO4)(2)O at 37.4 degrees C. *Calcified Tissue International* 60 (6).

Mondini, C., M. L. Cayuela, T. Sinicco, M. A. Sanchez-Monedero, E. Bertolone, and L. Bardi. 2008. Soil application of meat and bone meal. Short-term effects on mineralization dynamics and soil biochemical and microbiological properties. *Soil Biology & Biochemistry* 40 (2).

Montastruc, L., C. Azzaro-Pantel, B. Biscans, M. Cabassud, and S. Domenech. 2003. A thermochemical approach for calcium phosphate precipitation modeling in a pellet reactor. *Chemical Engineering Journal* 94 (1).

Moreno, E. C., T. M. Gregory, and W. E. Brown. 1968. Preparation and solubility of hydroxyapatite. *Journal of Research of the National Bureau of Standards Section a-Physics and Chemistry* A 72 (6).
Moreno, E. C., W. E. Brown, and G. Osborn. 1960. Stability of dicalcium phosphate dihydrate in aqueous solutions and solubility of octocalcium phosphate. *Soil Science Society of America Journal* 24 (2).
Murphy, J., and J. P. Riley. 1962. A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta* 26 (1).
Nelson, N. O., S. C. Agudelo, W. Q. Yuan, and J. Gan. 2011. Nitrogen and Phosphorus Availability in Biochar-Amended Soils. *Soil Science* 176 (5).
Neset, T. S. S., and D. Cordell. 2012. Global phosphorus scarcity: identifying synergies for a sustainable future. *Journal of the Science of Food and Agriculture* 92 (1):2-6.
Novotny, E. H. , R. Auccaise, M. H. R. Velloso, et al. 2012. Characterization of phosphate structures in biochar from swine bones. *Pesquisa Agropecuaria Brasileira* 47 (5).
Peak, D., J. T. Sims, and D. L. Sparks. 2002. Solid-state speciation of natural and alum-amended poultry litter using XANES spectroscopy. *Environmental Science & Technology* 36 (20).
Posner, A. S. 1985. The structure of bone apatite surfaces. *Journal of Biomedical Materials Research* 19 (3).
Raison, R. J., P. K. Khanna, and P. V. Woods. 1985. Mechanisms of element transfer to the atmosphere during vegetation fires. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* 15 (1).
Ravel, B., and M. Newville. 2005. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. *Journal of Synchrotron Radiation* 12.
Sato, S., D. Solomon, C. Hyland, Q. M. Ketterings, and J. Lehmann. 2005. Phosphorus speciation in manure and manure-amended soils using XANES spectroscopy. *Environmental Science & Technology* 39 (19).
Smil, V. 2000. Phosphorus in the environment: Natural flows and human interferences. *Annual Review of Energy and the Environment* 25.
Tilman, D., J. Fargione, B. Wolff, et al. 2001. Forecasting agriculturally driven global environmental change. *Science* 292 (5515).
Toor, G. S., J. D. Peak, and J. T. Sims. 2005. Phosphorus speciation in broiler litter and turkey manure produced from modiried diets. *Journal of Environmental Quality* 34 (2).
Vaccari, David A. 2009. Phosphorus: A Looming Crisis. *Scientific American* 300 (6).
Wang, Tao, Marta Camps-Arbestain, Mike Hedley, and Peter Bishop. 2012. Predicting phosphorus bioavailability from high-ash biochars. *Plant and Soil* 357 (1-2).
Warren, G. P., J. S. Robinson, and E. Someus. 2009. Dissolution of phosphorus from animal bone char in 12 soils. *Nutrient Cycling in Agroecosystems* 84 (2).
Wopenka, B., and J. D. Pasteris. 2005. A mineralogical perspective on the apatite in bone. *Materials Science & Engineering C-Biomimetic and Supramolecular Systems* 25 (2).
Ylivainio, K., R. Uusitalo, and E. Turtola. 2008. Meat bone meal and fox manure as P sources for ryegrass (Lolium multiflorum) grown on a limed soil. *Nutrient Cycling in Agroecosystems* 81 (3).

CHAPTER 2

PHOSPHORUS AVAILABILITY FROM RENDERED BONE CHAR IN AN ACID, PHOSPHORUS-FIXING SOIL INFLUENCED BY WOOD BIOCHAR AND SOIL EXPLORATION CAPACITY OF MAIZE (ZEA MAYS, L.)[2]

Abstract

Phosphorus (P) is an immobile nutrient and is especially challenging to manage in highly weathered, acid soils that are prone to strong P adsorption. With the rapid depletion of global P reserves, finding sustainable solutions to optimize P availability in agricultural ecosystems will become even more important. This study tests the P fertilizer potential of rendered bone char pyrolyzed at 350ºC and 750ºC, evaluates the effects of wood biochar on P availability in an Ethiopian, acid soil and determines the role of maize root (*Zea mays* L., variety B73) soil exploration capacity with and without root hairs or associated with arbuscular mycorrhizae (AM) in its ability to access P from sources with varying solubility. Results from an abiotic incubation focusing on soil chemical interactions found that pyrolyzing rendered bone at 350°C led to a 67% increase in resin-P. The addition of wood biochar applied before or after pyrolysis decreased resin-P by 14-26%, suggesting either increased weak P sorption to biochar, removal of low molecular weight organic acids or sequestration in biochar pores leading to long diffusive paths. The 13-19% increase in Olsen-P from co-pyrolyzed rendered bone and wood biochar may be explained by a reduction in CaP crystal formation by wood biomass during charring and may indicate that any P adsorption by the biochar was easily reversible. A five-week pot experiment

[2] To be submitted as a revised version to a journal under Zwetsloot, M. J.; Lehmann, J.; Bauerle, T.

with maize demonstrated that maize associated with AM had similar access to P from rendered bone char and Triple Superphosphate (TSP) fertilizer. Root branching may have been used as a strategy by maize to enhance AM infection rather than to increase root P uptake. Maize associated with AM showed lower total P accumulation when wood was added to the rendered bone before charring. Future research should concentrate on testing the P availability from bone char in different soil environments and unraveling the mechanism by which wood biochar reduces P accumulation when maize roots are inoculated with AM.

Introduction

Managing phosphorus (P) availability and understanding P dynamics in soils is essential to sustain agricultural growth worldwide. Soil P immobility (Sanchez 1976; Marschner 1995, Hinsinger 2001, Raghothama and Karthikeyan 2005) results in management challenges especially in weathered, acid soils that are prone to P-fixation (Sanchez 1976; Haynes and Mokolobate 2001). Rapidly depleting global P reserves (Abelson 1999; Smil 2000), call for alternatives to rock phosphate-dependent fertilizers and sustainable management practices that optimize P availability in agro-ecosystems(Cordell et al. 2009; Cordell et al. 2011).

Generating P fertilizer from household and industrial waste is one way to recycle P back into agricultural systems (Neset and Cordell 2012). Slaughterhouse waste is rich in calcium phosphates, but many of its uses are banned because of the risk of Bovine Spongiform Encephalopathy (BSE) associated with animal waste (Cascarosa et al. 2012). Using bone and meat residue to produce bone char through the process of pyrolysis may be able to overcome this problem, as BSE pathogens are routinely destroyed at 133°C for 20 min at 3 bar (Kingombe et al. 2001) or by combustion at 850°C for at least 2 seconds (Ayllon et al. 2006).

Bone mainly consists of biological apatite, a relatively crystalline calcium phosphate structure (Wopenka and Pasteris 2003; Wopenka and Pasteris 2005); however, less is known about its characteristics and P fertilizer efficacy after pyrolysis. Incubation studies have found that bone char produced at 400°C was a more effective P fertilizer than GAFSA rock phosphate (Warren et al. 2009). However, both increases and decreases in dry matter biomass and P concentration were observed when this same bone char was used as P fertilizer for potato, onion and wheat crops (Siebers et al. 2012). This warrants further investigation on plant-available P from bone char and how it compares to more soluble sources of P such as Triple Super Phosphate (TSP) fertilizer. Moreover, it still unknown how P availability from bone char applied to soil is affected by its production temperature and biomass additions or co-pyrolysis with biochar.

P-fixing soils are common in tropical farming systems (Sanchez 1976). Their high mineral oxide content chemisorbs phosphate from the soil solution (Parfitt et al. 1975), making applied P fertilizers less plant-available. Organic matter additions to P-fixing soils have shown to decrease P adsorption and increase P desorption (Singh and Jones 1976; Guppy et al. 2005). The same has been observed for biochar additions: an adsorption isotherm study showed that biochar amendments can reduce P adsorption and increase P desorption from ferrihydrite (Cui et al. 2011). The presence of biochar in Ferralsol and Anthrosol soils increased total plant P uptake when P fertilizer was added (Lehmann et al. 2003). On the other hand, without the addition of sufficient P, biochar reduced Mehlich-3 extractable P (Nelson et al. 2011). The biochar properties and mechanisms that are responsible for a decrease or increase in P availability are still unclear.

In addition to innovative ways in managing P inputs, plant biologists are advocating for a different solution to overcome low plant P nutrition including breeding plants with root system architecture that are better capable of mining for P (Lynch 2007). Moreover, recent research has highlighted the importance of root foraging (Ramaekers et al. 2010; Richardson et al. 2011); increased root hair elongation, top soil lateral branching, high root:shoot ratios, exudation of low molecular weight organic acids, protons and enzymes, and increased root P uptake kinetics are all found to be strategies that favor P use efficiency (Lynch and Beebe 1995; Bates and Lynch 2001; Vance et al. 2003; Hodge et al. 2009; Ramaekers et al. 2010; Zhu et al. 2010; Lynch 2011). In plants associated with arbuscular mycorrhizae (AM), these adaptations may be less important to enhance P acquisition (Vance et al. 2003).

There are very few studies that simultaneously examine soil P availability and plant root P acquisition, and their interaction. While many have investigated root architecture differences in response to P-deficient environments and to concentrated nutrient patches, no information about root foraging behavior in response to soil available P sources with distinct chemical characteristics has been published (Hodge 2006). Moreover, it is unknown how plants with different root foraging strategies access soil P sources with varying solubility.

The objectives of this study were: (1) to test the potential of bone char as a P fertilizer in comparison to TSP fertilizer; (2) to examine the effect of co-pyrolyzed biochar on the P availability from bone char in a P-fixing soil; (3) to evaluate how maize roots with differing root architecture as influenced by the presence/absence of root hairs and AM associations make use of soil P sources of varying solubility.

Materials and Methods

Soil

Soil was collected from a research farm at Jimma University in Jimma, Ethiopia. Olsen-extractable P after a five-week incubation was 3.08 mg P kg soil^{-1}. Soil pH was 4.51 in water and 3.97 in 1 M KCL. Cation exchange capacity was 279.1 mmole kg soil^{-1} and contained exchangeable base cations at levels of 5.22 g Ca kg^{-1} soil, 2.90 g K kg^{-1} soil, 1.20 g Mg kg^{-1} soil, 0.06 g Na kg^{-1} soil. Total C was 3.1% and total N 0.27%. Langmuir adsorption isotherm showed substantial P sorption with a maximum sorption capacity of 456 mg P kg^{-1} soil. Soil texture was 50% clay, 40% silt and 10% sand.

Preparation of Phosphorus Sources

Rendered bone meal was purchased from The Espoma Company 1929 (Milville, NJ, USA). Robinson Lumber in Owego, NY, USA supplied hard wood chips (80% red maple, 20% sugar maple). Wood chips were oven-dried at 60°C and ground to a particle size < 2mm with a Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA). Rendered bone meal was pyrolyzed at a heating rate of 2.5 °C min^{-1} and temperature was maintained at 350 and 750°C for 45 min in a muffle furnace swept with argon gas. Rendered bone meal mixed with hard wood chips < 2 mm at a dry weight ratio of 1:1 were charred under the same pyrolysis conditions. The pyrolyzed bone and rendered bone-wood char were ground with a mortar and pestle to a particle size of 74-150 µm. The original rendered bone meal and dried wood chips were difficult to grind with mortar and pestle and were therefore ground with a Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) to mesh size 60.

Greenkeeper's Secret Triple Superphosphate (TSP) fertilizer (T&N, Inc., Foristell, MO, USA) was used as proxy for a soluble P source. To locate TSP in wood char pores, TSP was dissolved in water and shaken with wood char produced at 350°C or 750°C in a centrifuge tube for 24 hrs. The centrifuge tube containing dissolved TSP and wood char was dried at 60°C for 4 days before soil application.

Abiotic Incubation Design

Thirteen different P sources (Table 2.1) were tested including rendered bone meal, rendered bone char pyrolyzed at 350°C and 750°C, a mixture of separately charred rendered bone and wood at 60°C, 350°C and 750°C, rendered bone-wood char pyrolyzed together at 350°C and 750°C, TSP alone, TSP and wood biochar at 350°C and 750°C separately added to the same jar, TSP dissolved into pores of wood biochar at 350°C and 750°C. These were compared to unamended controls and wood (char) at 60°C, 350°C and 750°C. These chars were also used in previous study for analysis of P chemical characteristics (Zwetsloot et al. unpublished). Each treatment was replicated five times.

The treatments were applied to 250 mL mason jars containing 5 g of soil sieved to < 2 mm. Deionized water was added to saturation (4 mL jar^{-1}). To prevent microbial activity, $HgCl_2$ was dissolved in the deionized water prior to application at a rate of 500 mg $HgCl_2$ kg^{-1} soil (Tuominen et al. 1994). Bone and TSP fertilizers were applied at a rate of 360 mg P kg^{-1} soil, which was based on 80% of the maximum sorption capacity of soil. All jars were put on a rotary shaker at 25 rpm at 30°C for 5 weeks.

Table 2.1: Abiotic incubation treatments.

Phosphorus Source Treatments	**Control Treatments**
Rendered bone meal	No Addition
Rendered bone char 350°C	
Rendered bone char 750°C	
Rendered bone meal + wood biomass 60°C	Wood biomass 60°C
Rendered bone char 350°C + wood biochar 350°C	Wood biochar 350°C
Rendered bone char 750°C + wood biochar 750°C	Wood biochar 750°C
Rendered bone-wood char 350	
Rendered bone-wood char 750	
TSP + wood biomass 60°C	
TSP + wood biochar 350°C	
TSP + wood biochar 750°C	
TSP in wood biochar pores 350°C	
TSP in wood biochar pores 750°C	

Abiotic Incubation Analyses

Total contents of the jars (5 g + treatment) were used for analysis. In the case of Olsen-P, 100 mL of 0.5 M $NaHCO_3$ adjusted to pH = 8.5 with 1 M NaOH solution was added to the samples and put on a horizontal shaker at 100 rpm for 30 min (Kuo 1996). After filtering with both a 2V Whatman qualitative filter paper and 0.45 µm filter paper, the filtrates received 3.25 mL 12 M HCl to lower the pH for colorimetric P determination (Murphy and Riley 1962).

The resin-P method was slightly modified from the first step of the sequential P fraction of Tiessen and Moir (1993). Anion-exchange resin (AER) strips (Ionac MA-7500, LANXESS Sybron, Birmingham, NJ, USA) were cut to 20 by 60 mm strips, rinsed four times with deionized

water, soaked in 0.5 M $NaHCO_3$ (25 strips L^{-1}) overnight, and finally rinsed with and soaked in deionized water before use. One AER strip together with 150 mL deionized water was added to each jar and placed on a rotary shaker at 100 rpm for 24 hrs. AER strips were removed from the jars and placed in centrifuge tubes containing 40 mL 0.5 M HCl. After shaking the centrifuge tubes at 200 rpm for 20 hrs, AER strips were taken out and 0.5 M HCL extracts were colorimetrically analyzed for P (Murphy and Riley 1962). Olsen-P and Resin-P extracts from the TSP treatments were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES Thermo Jarrel Ash 166 Trace Analyzer, Thermo Jarrell Ash Corporation, Franklin, MA, USA).

Pot Trial Design

The pot trial followed a completely randomized block design with two factors P source and soil exploration capacity (Figure 2.1). The P source treatments included TSP fertilizer, rendered bone char 750°C, rendered bone-wood char 750°C and no P application, which were sieved to < 2 mm before soil application. Maize (*Zea mays,* L.) variety B73 was supplied by USDA Genetic Resource Information Network in Beltsville, MD, USA. All three soil exploration treatments used the same maize variety B73, but their efficiency to take up P was altered. A rth1-1 mutant in B73 (115A) (Wen and Schnable 1994, Hochholdinger et al. 2008)was ordered through the Maize Genetics Coop Stock Center (Urbana, IL, USA).. B73 variety by itself functioned as the root hair (RH) factor. For the root hair and arbuscular mycorrhizae (RH + AM) factor, the B73 maize was inoculated with *Glomus clarum* (strain WV325, INVAM, West Virginia University, USA).

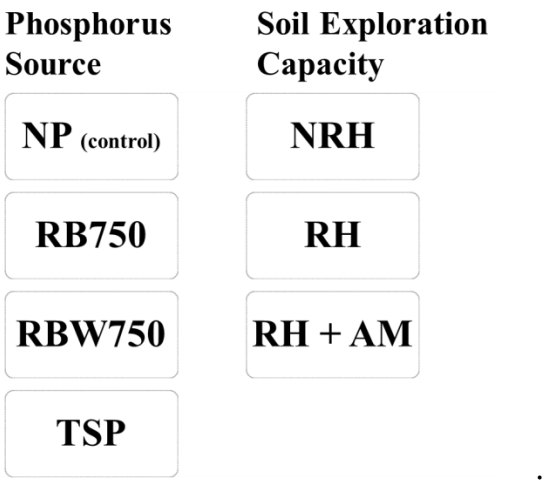

Figure 2.1: Experimental design treatments including no P application (NP), rendered bone char produced at 750°C, co-pyrolyzed rendered bone-wood char produced at 750°C and Triple Superphosphate (TSP) fertilizer for P source applications and no root hairs (NRH), root hairs (RH) and roots associated with AM (RH + AM) for maize root soil exploration capacity.

All twelve treatments were replicated five times. One set of a single replicate of each treatment was planted per day in a 2.40 m by 1.35 m growth chamber at the Guterman Bioclimatic Laboratory and Greenhouse Complex at Cornell University, Ithaca, NY, USA. Model CP512 tree pots (volume = 5 L, height = 0.305 m, width = 127 mm) were purchased from Stuewe and Sons Inc (Tangent, OR, USA). P sources were added at a rate of 100 P kg ha^{-1}, which is equivalent to 161.3 mg P pot^{-1}. Since bone and wood biomass were co-pyrolyzed, this application rate determined the wood biochar application in the rendered bone-wood char treatment, which amounted to 0.01% by soil weight. Pots were filled with 4 L of soil and consisted of three layers (supporting information Figure-S1). The 650 mL top and 2100 mL bottom layer contained soil and a P source. The *G. clarum* or control treatments were incorporated into the 1250-mL mid layer of the soil and P source at a volume ratio of 1:25. Inoculant carrier material was Agsorb attapulgite (Oildri, Chicago, IL, USA) with dried roots and spores from a sorghum-sundangrass pot culture with low P. As control, carrier material without

inoculant was added. Three B73 maize seeds were placed 2 inches above the middle layer. Three days after germination, the two weakest seedlings were removed. Limited by the amount of seeds, the NRH treatment only received one B73 115A seed per pot.

On even days, pots were watered by weight to 50% water filled pore space and root production, growth and death were tracked on the pot windows with colored markers for root survivorship analysis. On odd days, plants received 100 mL of nutrient solution:1.5 mM $Ca(NO_3)_2$, 5 mM NH_4NO_3, 3 mM KNO_3, 2 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.024 mM Fe-EDTA, 0.05 mM KCL, 0.025 mM H_3BO_4, 0.007 mM $MnSO_4$, 0.006 mM $ZnSO_4$, 0.002 mM $CuSO_4$ and 0.0005 mM $(NH_4)_6Mo_7O_{24}$. On day 10, all pots received an additional 100 mL of 15 mM K_2SO_4. Duration of seeding to harvest was five weeks.

Pot Trial Analyses

At harvest, shoots were cut and dried at 60°C for 4 days. Fresh and dry weights (g) were measured. Pots with soil and roots were vertically sliced in two halves. One half was carefully washed with water and sampled for intact crown roots (adventitious or shoot-born roots) that were used for morphological analyses. Sampled roots were immediately stained with a 0.167 g L^{-1} neutral red dye solution. Soil from the other half of the pot was sieved to 4 mm. Soil samples were taken for chemical analyses. All remaining roots were washed with water and dried at 60°C for 4 days after which root dry weight (g) was measured.

To determine plant P concentration and total P accumulation, both shoot and root biomass were digested in a mixture of NHO_3 and 30% H_2O_2 (Benton Jones 2001) and digests were submitted for inductively coupled plasma atomic emission spectrometry (ICP-AES Thermo Jarrell Ash 166 Trace Analyzer, Thermo Jarrell Ash Corporation, Franklin, MA).

Crown roots dyed with neutral red were scanned. Software package WinRHIZO Pro 2007d (Regent Instruments Inc.) and data for number and proportion of root orders, root surface area, root length, average root diameter of first-order roots (supporting information Figure-S2). As first-order roots are responsible for nutrient uptake and are the first to show morphological changes in response to nutrient patches (Sattelmacher et al. 1993), analysis mainly focused on the morphological characteristics of first-order roots. Root survivorship analysis was not performed because no root death was observed during the experiment.

Statistical Analyses

JMP Pro 10 was used for all statistical analyses (SAS, Cary, NC, USA). Student's t-tests determined differences between treatments. Chi-square goodness-of-fit test estimated differences in number of roots orders among treatments. A linear regression between resin-P and Olsen-P, total plant P accumulation and resin-P, and total plant P accumulation and Olsen-P established the effectiveness of the abiotic incubation extraction methods to predict total plant P accumulation. Total plant biomass, total plant P accumulation, root P accumulation, total plant calcium (Ca) accumulation, average length of first-order roots, and average surface of first-order roots were transformed with natural logarithm before statistical analyses.

Results

Incubation

Olsen-P extractions showed that pyrolyzing bone or increasing pyrolysis temperature decreases extractable P ($P < 0.05$) from rendered bone (Figure 2.2A). Individually adding wood biochar to the bone char or TSP fertilizers did not affect Olsen-P ($P > 0.05$). Co-pyrolyzing

rendered bone and wood at 350°C and 750°C, however, led to a 13-19% increase in Olsen-P ($P < 0.05$). Shaking TSP and wood biochar in water and letting it dry before addition to soil decreased rather than increased Olsen-P by 22-36% ($P < 0.05$). Olsen-P from uncharred rendered bone and rendered bone char at 350°C was 14-28% higher than Olsen-P from TSP ($P < 0.05$). There was no difference in Olsen-P between TSP and rendered bone char at 750°C.

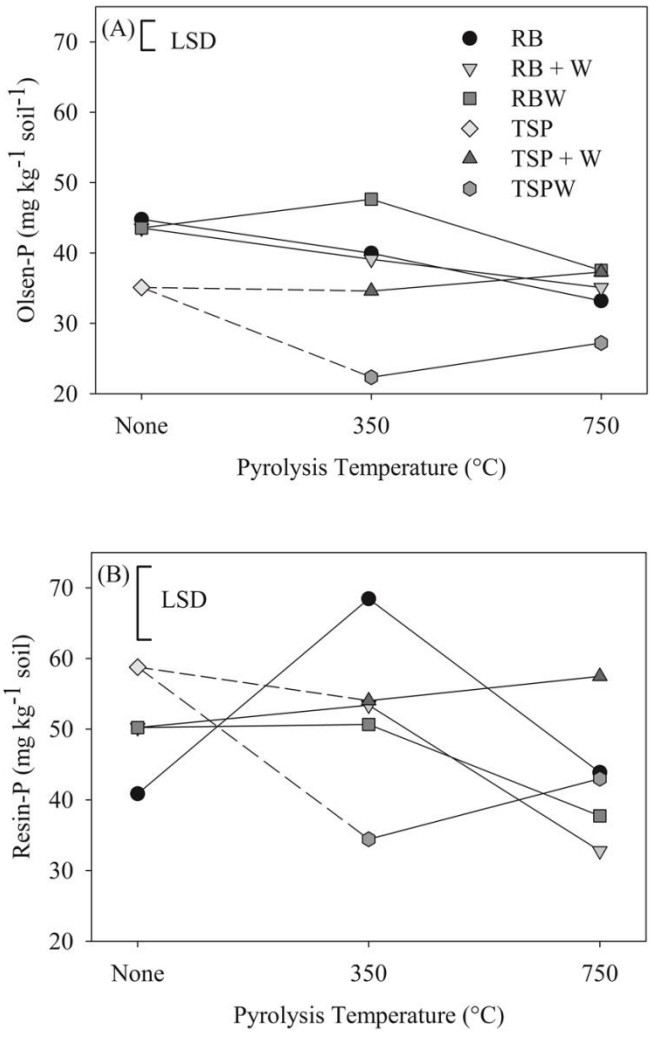

Figure 2.2: (A) Olsen- and (B) Resin-extractable P from P-fixing soil after a five-week incubation with different P sources and wood additions dried or pyrolyzed at 350°C and 750°C. Treatments include rendered bone (RB), rendered bone and wood individually added (RB+W), rendered bone and wood mixed before pyrolysis (RBW), Triple Super Phosphate (TSP) fertilizer, TSP and wood biochar individually added (TSP+W), TSP dissolved in wood biochar pores (TSPW) (n=5; LSD is the least significant difference at $P<0.05$).

Resin-P of incubated soil and P sources were only weakly correlated with Olsen-P (R^2 = 0.13, $P < 0.05$, Table 2.2). Pyrolyzing rendered bone at 350°C resulted in 67% higher resin-P compared to uncharred bone ($P < 0.05$, Figure 2.2B). However, this effect disappeared at 750°C, which had a similar resin-P to the unpyrolyzed rendered bone ($P > 0.05$). The separate addition of wood biochar and co-pyrolysis of rendered bone and wood decreased resin-P by 14-26% in comparison to rendered bone char alone ($P < 0.05$). Adding fresh biomass to the uncharred rendered bone led to a 23% increase in resin-P ($P < 0.05$). However, for the more soluble P source TSP, shaking wood biochar with TSP before application reduced resin-P by 27-41%. With R^2=0.80, resin-P was a better predictor for P accumulation in maize than Olsen-P, for which R^2=0.52 (Table 2.2).

Table 2.2: Linear regression results between resin-P and Olsen-P, plant P accumulation and resin-P, and plant P accumulation and Olsen-P. * Only P accumulation results of maize RH treatment were used.

Dependent variable	**Independent variable**	**Parameter estimate**	**R^2**	**P-value**	**Sample size**
Resin-P	Olsen-P	0.61	0.13	0.0039	65
Olsen-P	Resin-P	0.21	0.13	0.0039	65
Plant P accumulation	Resin-P	0.02	0.80	< 0.0001	16*
Plant P accumulation	Olsen-P	0.03	0.52	0.017	16*

Pot trial

Increasing soil exploration capacity of maize (NRH → RH → RH + AM) increased total plant biomass ($P < 0.05$) for all P sources (Figure 2.3A). Without P additions, total biomass did not differ regardless of whether the maize had root hairs or not ($P > 0.05$). However, inoculating maize that had root hairs with AM increased total plant biomass even without P additions

($P < 0.05$), albeit only by 172% compared to an increase by 378-396% when any P source was applied.

TSP application resulted in the highest total plant biomass for both maize with root hairs and inoculated with AM ($P < 0.05$). When maize was inoculated with AM, rendered bone char and rendered bone-wood char yielded the same biomass. However, maize with only root hairs had higher total biomass when fertilized by rendered bone char than by rendered bone-wood char ($P < 0.05$). Maize did not respond to P additions when it did not have root hairs ($P > 0.05$).

The root:shoot ratios of both NRH and RH+AM were significantly higher than those of RH ($P < 0.05$, Figure 2.3B), but did not differ between NRH and RH + AM ($P > 0.05$). The root:shoot ratios after rendered bone char additions were greater than those after additions of TSP when maize was inoculated with AM ($P > 0.05$). Without P application root:shoot ratios were significantly lower than when other P sources were applied ($P < 0.05$), except after inoculation with AM ($P > 0.05$).

Total plant P accumulation, root P accumulation and shoot P accumulation followed similar trends as total plant biomass (Figure 2.3C, supporting information Figure 2.S3) with the following exceptions: P additions did not result in increased P accumulation when plants had no root hairs; increases in P accumulation for plants with root hairs were lower than increases in biomass (no increase without P and with rendered-bone wood char, $P > 0.05$) and with AM plant P accumulation was not different between rendered bone char and TSP ($P > 0.05$), while mixing with wood biochar significantly lowered total P accumulation ($P < 0.05$).

Total plant Ca accumulation increased with improved soil exploration capacity of the maize plants (Figure 2.3F). Without root hairs, Ca accumulation did not differ among P sources ($P > 0.05$). When the maize had root hairs, fertilization with TSP demonstrated higher Ca

accumulation than the three other P sources ($P < 0.05$). Maize colonized with AM did not show significant differences in Ca accumulation between TSP and rendered bone char ($P > 0.05$), similar to plant P accumulation.

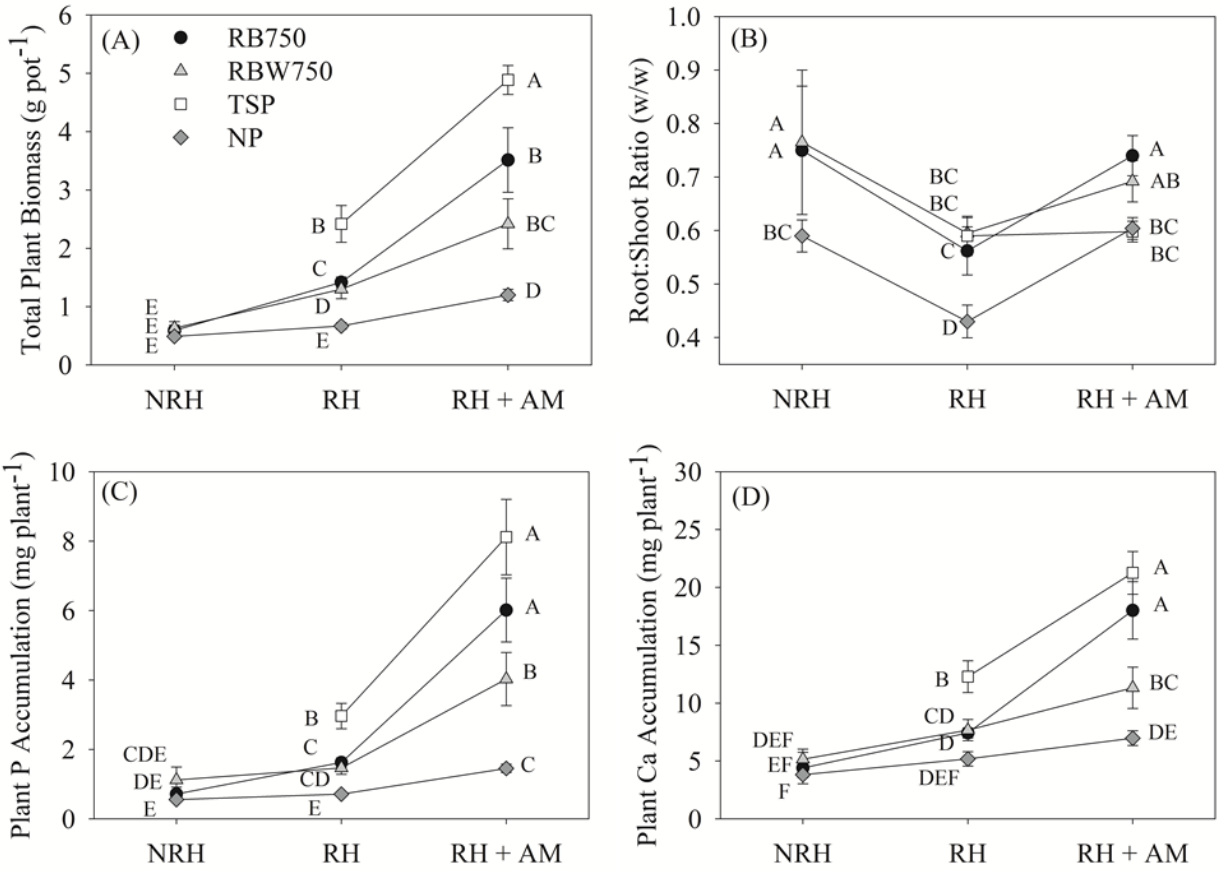

Figure 2.3: Pot trial harvest data after a five-week growing period of *Z. mays* (means and standard error, n = 5 except for NRH treatments where n = 2-3) fertilized with rendered bone char pyrolyzed at 750°C (RB750), rendered bone and wood pyrolyzed together at 750°C (RBW750), Triple Superphosphate (TSP) fertilizer, no P additions (NP): (A) total biomass, (B) root:shoot ratio, (C) root P accumulation and (D) shoot P accumulation, (E) root:shoot P accumulation ratio, and (F) total plant Ca accumulation. On the x-axis, soil exploration capacity of maize is altered through the absence of root hairs (NRH), the presence of root hairs (RH) and the presence of root hairs and addition of AM inoculants (RH + AM).

Plant and shoot P concentrations increased significantly with any P additions and were equally high with additions of bone or TSP fertilizers (Table 2.3). The greatest increase in plant P concentration by 12-51% was found when plants had AM in comparison to maize with only

root hairs. Root P concentration was lower for maize with root hairs in comparison to maize roots associated with AM when fertilized with rendered bone char, rendered bone-wood char and TSP ($P < 0.05$). In contrast, Ca concentrations were the highest for maize without root hairs or receiving no P application ($P < 0.05$), while Ca concentration for maize with RH + AM and TSP fertilizer was significantly lower than all other treatments ($P < 0.05$).

Table 2.3: P concentration of *Z. mays* total plant tissue, root and shoot, and Ca concentration of total plant tissue (means and standard deviation, n = 5 except for NRH treatments where n = 1-3). P sources include no P application (NP), rendered bone char 750°C (RB750), co-pyrolyzed rendered bone-wood char 750°C (RBW750) and Triple Super Phosphate fertilizer (TSP). Soil exploration capacity (SEC) of maize is altered through the absence of root hairs (NRH), the presence of (RH) and the presence of root hairs and addition of AM inoculants (RH + AM).

P source	SEC	Plant P ($mg\ g^{-1}$)	Plant Ca ($mg\ g^{-1}$)	Root P ($mg\ g^{-1}$)	Shoot P ($mg\ g^{-1}$)
NP	NRH	1.13 ± 0.07 ^{c}	7.84 ±0.43 ^{a}	1.04 ± 0.03 $^{b, c}$	1.19 ± 0.11 $^{b, c}$
NP	RH	1.08 ±0.08 ^{c}	7.72 ±0.47 ^{a}	1.02 ± 0.06 $^{b, c}$	1.10 ± 0.12 ^{c}
NP	RH + AM	1.21 ±0.04 $^{b, c}$	5.80 ±0.49 ^{b}	1.04 ± 0.17 $^{b, c}$	1.32 ± 0.06 $^{b, c}$
RB750	NRH	1.19 ±0.21 $^{a, b}$	7.27 ±1.67 ^{a}	1.18 ± 0.22 $^{a, b, c}$	1.33 ± 0.02 $^{b, c}$
RB750	RH	1.14 ±0.10 ^{c}	5.26 ±0.38 $^{b, c}$	0.86 ± 0.26 ^{c}	1.30 ± 0.06 $^{b, c}$
RB750	RH + AM	1.72 ±0.26 ^{a}	5.22 ±0.65 $^{b, c}$	1.50 ± 0.26 ^{a}	1.90 ± 0.39 ^{a}
RBW750	NRH	1.94 ±1.33 $^{b, c}$	8.67 ±4.12 $^{b, c}$	0.91 $^{b, c}$	1.12 ± 0.03 $^{b, c}$
RBW750	RH	1.13 ±0.06 ^{c}	5.92 ±0.36 ^{b}	0.85 ± 0.14 ^{c}	1.30 ± 0.06 $^{b, c}$
RBW750	RH + AM	1.63 ±0.18 ^{a}	4.81 ±0.45 $^{c, d}$	1.24 ± 0.27 ^{a,b}	1.92 ± 0.24 ^{a}
TSP	RH	1.23 ±0.10 $^{b, c}$	5.15 ±0.50 $^{b, c}$	0.84 ± 0.09 ^{c}	1.46 ± 0.09 ^{b}
TSP	RH + AM	1.70 ± 0.67 ^{a}	4.33 ±0.45 ^{d}	1.24 ± 0.47 $^{b, c}$	1.92 ± 1.16 ^{a}

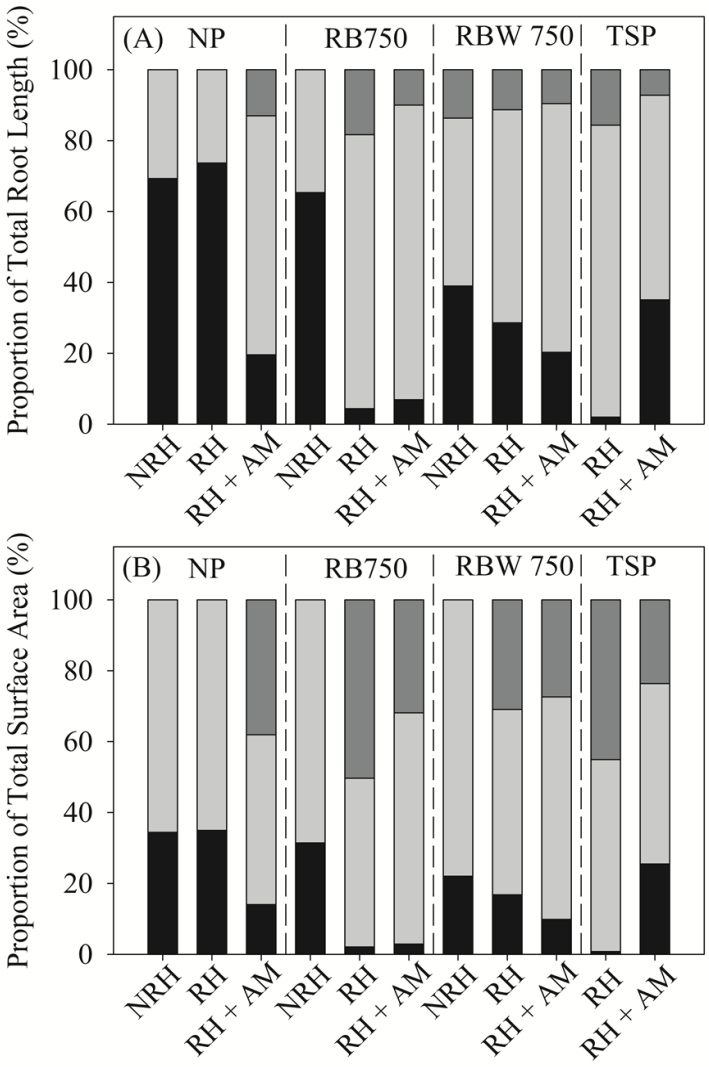

Figure 2.4: Root order distribution as proportion of (A) total length and (B) total surface area (means, n = 2-4. Grey scale defines different orders: black = order 1; light grey = order 2; dark grey = order 3. P sources include no P application (NP), rendered bone char 750°C (RB750), co-pyrolyzed rendered bone-wood char 750°C (RBW750) and Triple Superphosphate fertilizer (TSP). On the x-axis, soil exploration capacity of maize is altered through the absence of root hairs (NRH), the presence of (RH) and the presence of root hairs and addition of AM inoculants (RH + AM).

With an increase in soil exploration capacity (NRH → RH → RH + AM), root branching increased ($P < 0.05$), except for maize roots without root hairs fertilized with rendered bone-wood char that already had three root orders. The root order distribution data as proportion of

total length and total surface area were not normally distributed and could not be transformed. Nonparametric statistical tests were not significant due to large variation within treatments. However, trends in root order distribution influenced by soil exploration capacity and P source are evident. For rendered bone char and rendered bone-wood char, the presence of root hairs resulted in a lower proportion of first-order roots and higher percentage of second-order roots for both total length and total surface area (Figure 2.4). When no P was applied, no differences were found. Association with AM led to a decrease of the total root length and root surface proportions of first-order roots and an increase of the second-order roots when no P and rendered bone-wood char was applied, had no effect for rendered bone char additions, and increased for TSP. For rendered bone char and TSP fertilizer, presence of AM led to a higher number of first-order root tips compared to other treatments ($P < 0.05$, Figure 2.5A). Among maize with root hairs but no AM, TSP and rendered bone char application resulted in significantly more first-order root tips than rendered bone-wood char or no P application ($P < 0.05$). The average diameter of first-order roots did not change for all treatments ($P > 0.05$, Figure 2.5B).

The average length of first-order roots was significantly higher without P application in comparison to the rendered bone char and TSP additions with root hairs ($P < 0.05$, Figure 2.5C). The same trend held true for the average surface area of first-order roots when no P or TSP was applied ($P < 0.05$, Figure 2.5D).

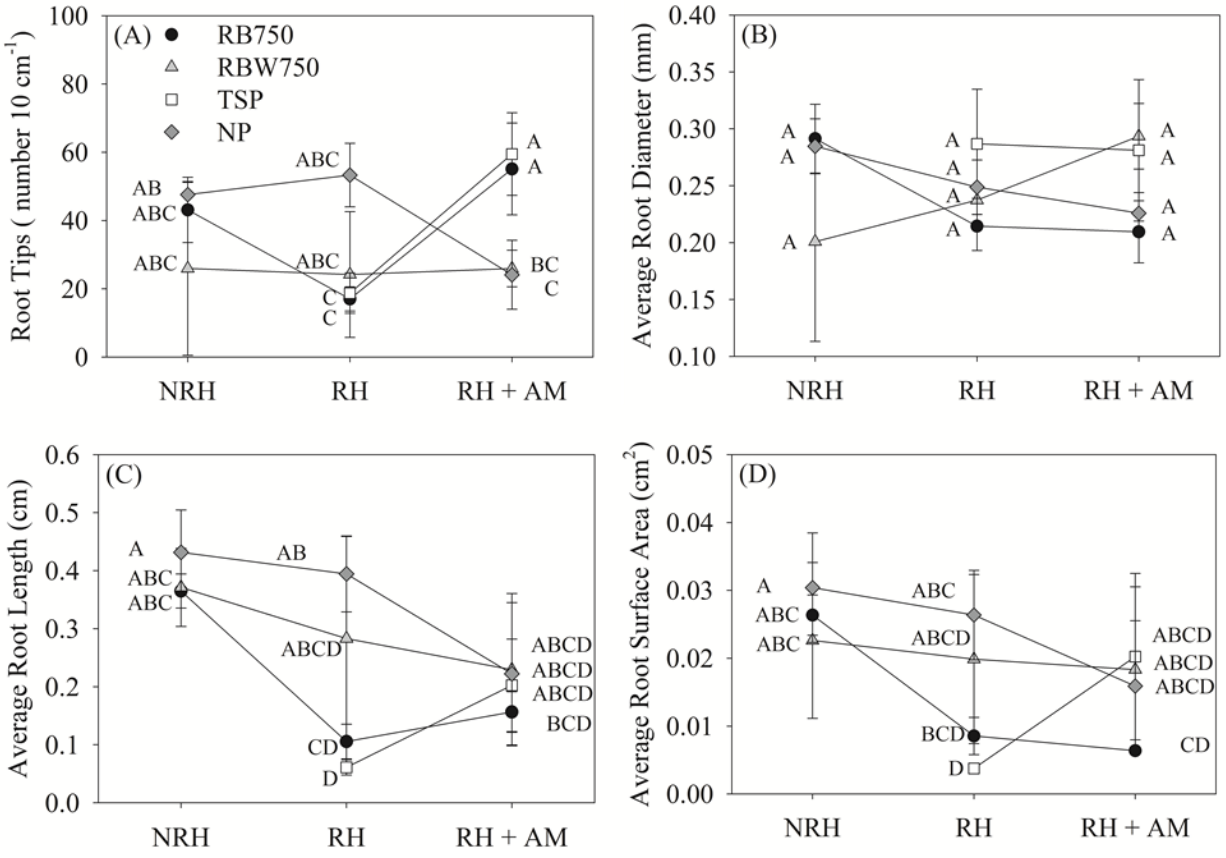

Figure 2.5: Morphological characteristics of first-order roots (means and standard error, n = 3 except for NRH treatments for which n = 2): (A) number of root tips, (B) average root diameter, (C) average root length, (D), average root surface area. P sources include no P application (NP), rendered bone char 750°C (RB750), co-pyrolyzed rendered bone-wood char 750°C (RBW750) and Triple Super Phosphate fertilizer (TSP). On the x-axis, soil exploration capacity of maize is altered through the absence of root hairs (NRH), the presence of (RH) and the presence of root hairs and addition of AM inoculants (RH + AM).

Discussion

P Fertilizer Characteristics of Bone Char

Rendered bone char is as effective in providing plant-available P as commercial TSP fertilizer in a highly weathered, acid soil. Both resin-P and Olsen-P from soil incubated with rendered bone char produced at 350°C even indicated a higher P availability than when soils

were incubated with TSP. This may be explained by the ability of the roots and AM to take up highly soluble P such as from TSP before it is strongly adsorbed to soil minerals.

Unlike more crystalline P sources such as rock phosphate and bone char, TSP is highly water-soluble and becomes immediately plant-available. Over time it becomes increasingly less available to plant roots through adsorption reactions with the soil surfaces (Kucey et al. 1989), especially in acid soils. High soil acidity facilitates the continued dissolution of apatite through the release of structural OH^- (Rajan et al. 1996). The results from this experiment may therefore not be applicable to neutral or basic soils.

The greater resin-P after additions of rendered bone char than of uncharred bone meal can likely be explained by the increase in CaP crystal formation at higher pyrolysis temperatures, which reduces water-P but increases formic-P representing plant-available P (Zwetsloot et al., unpublished). At higher CaP crystallinity, calcium phosphate in bone starts to resemble hydroxyapatite (HA) more closely. HA is more soluble than other calcium phosphates in solutions with low pH (Brown et al. 1975; Matsumoto et al. 2002), improving P availability from bone char in acid soil environments. In addition, pyrolysis frees up the organic bone constituents between 250 and 500°C (Deydier et al. 2005). Cleaving organic P bonds could enhance extractable P (DeLuca et al. 2009). At pyrolysis temperatures higher than 500°C, free P from organic bone constituents may become less available again through its incorporation into HA crystal lattice structure. Rendered bone char produced at 750°C has indeed lower resin-P than rendered bone char produced at 350°C.

The reduction in Olsen-P with an increase in pyrolysis temperature can also be explained by an increase in CaP crystallinity. While the OH^- anions from the Olsen solution (pH = 8.5) increase P solubility through complexing or precipitating Al^{3+} and Fe^{2+}, the increase in soil

solution pH might have limited the dissolution of formic-P in calcium phosphate sources. Hence, rather than extracting the formic-extractable P fraction, Olsen-P relied more on the water-P fraction of rendered bone char, which decreases with CaP crystallization. Likewise, the increase in Olsen-P when rendered bone is charred together with wood biomass is likely attributed to a decrease in CaP crystal formation and increase in water-P (Zwetsloot et al., unpublished) rather than a reduction in soil P-fixation by biochar.

Fertilization of maize with rendered bone char led to a significantly higher Ca tissue concentration than with TSP, a result of the higher Ca content and Ca:P ratio in rendered bone char (supporting information Table S1.1).

Biochar Additions

Mixing the tested wood biochar with a P source before or after co-pyrolysis did not increase P availability. The decrease in resin-P from soil incubated with biochar together with TSP or bone fertilizers demonstrates that part of this observation can be explained by an interaction between the soil and biochar. Biochar contains ash minerals that can precipitate P out of solution (Hollister et al. 2013) and can initially have limited anion-exchange creating sites for P sorption (Cheng et al. 2008), which could both have reduced P availability. This is not a likely explanation as Olson-P actually increased.

In addition, biochar may have served as an adsorbent for organic acids already present in the soil (Cornelissen et al. 2005). Low molecular weight organic acids from root exudates and dead plant material can decrease P-fixation through competition for adsorption surfaces or complexing with Fe^{2+} and Al^{3+}, thereby increasing the P concentration in the soil solution (Bolan et al. 1994; Jones 1998; Hinsinger 2001; Antelo et al. 2007). If the wood biochar removed

organic acids from the soil solution, the opportunity for P-fixation may have increased. In addition, Gerke and Hermann (1992) found that soil organic matter can also increase P adsorption since organo-Fe complexes may have a higher P sorption capacity than inorganic Fe- and Al-oxide surface. If biochar complexed Fe^{2+} and Al^{3+}, it could have increased the P sorption capacity of the soil. Further research should determine which mechanism is most prevalent.

Wood biochar may have also decreased the efficacy of the AM to colonize maize roots or the capacity of AM to mine for P. Both increases and decreases in AM abundance after biochar application have been reported (Warnock et al. 2007; Lehmann et al. 2011). Previous research demonstrates that activated carbon or biochar has capacity to adsorb signaling compounds from roots and AM. If this process is irreversible, this could reduce the capacity of AM to colonize roots (Warnock et al. 2007). For example, wood biochar could have adsorbed strigolactones exuded by plants to stimulate hyphal branching (Akiyama et al. 2005).

Pyrolyzing the wood biomass may have reduced the amount of low molecular weight organic acids that may otherwise decrease P adsorption from added unpyrolyzed wood. A higher concentration of low molecular weight organic acids would increase the competition with P for mineral oxide surfaces and decrease P adsorption (Earl et al. 1979; Haynes and Mokolobate 2001; Hunt et al. 2007). The addition of dry wood biomass increased resin-P from unpyrolyzed bone meal, suggesting that the biomass source provided an extra supply of low molecular weight organic acids that reduced P-fixation. In contrast, wood biochar additions did not change or decreased resin-P.

Soil Exploration Capacity

Maize roots colonized by AM are equally capable in acquiring P from sources with varying solubility, unlike maize root systems with only root hairs which had limited access to P from more crystalline rendered bone char. AM inoculation would have increased the soil volume explored for P. As rendered bone char was less evenly distributed through the soil than TSP that evenly dissolves into the soil solution, efficient P acquisition from rendered bone char may require better soil exploration. Moreover, previous studies have found an almost threefold increase in P release from apatite under mycorrhizal colonization, suggesting fungal facilitation of mineral weathering (Smits et al. 2012). This could have increased P solubility of rendered bone char – which has an apatite-like CaP mineral structure (Zwetsloot et al., unpublished) – under AM inoculation and explains how differences in plant P accumulation between P sources with varying solubility were smaller with than without AM.

Building first-order roots with increased length and surface area was used as strategy to increase P uptake under low P availability. Longer first-order laterals provide greater absorptive surface for nutrient uptake, but have a higher cost for the transportation of nutrients (Fitter 1991). Only when P was severely limited, the benefits of having long first-order roots for increased P uptake outweighed the costs. This was the case with applications of rendered bone-wood char or without P when not inoculated with AM. These plants showed lower P accumulation, longer first-order roots and greater first-order root surface area than when TSP or rendered bone char was applied.

Root branching rather appeared to be a strategy to enhance AM infection than P uptake by roots. Maize roots associated with AM had more root orders and resulted in a more dichotomous root system. Moreover, AM inoculants increased the number of first-order root tips

in maize fertilized with rendered bone char or TSP in comparison to maize without AM associations. While root topology including root branching is very important for uptake of mobile nutrients, uptake of the comparatively immobile P may be more dependent on AM associations because of its narrow depletion zone around the root (Fitter 1991). Previous studies have also reported increased branching in response to AM colonization (Berta et al. 1993; Kaldorf and Ludwig-Muller 2000).

Co-pyrolysis of wood with bone reduced the ability of the AM-inoculated maize to access P. Interference of wood biochar in the plant-fungus signaling pathways may be an explanation (Vierheilig 2004). Maize plants with additions of rendered bone-wood char did not show an increase in the number of first-order root tips under AM colonization. A higher degree of branching could be a plant strategy to increase chances of AM infection. While the exact interactions between root and AM fungi prior to infection are still unclear, we can speculate that the root systems would have been less stimulated to increase branching if they could not detect the presence of *G. clarum*.

Conclusion

Charring was shown as a viable strategy to improve P availability from bones and bone char proved to be as effective for P fertilization as commercial TSP fertilizer in a high-P fixing soil. Co-pyrolyzing wood with bones, however, rather decreased P uptake by maize, despite a lower crystallinity after pyrolysis and greater extractability using Olsen soil test. The reasons may include precipitation with ash minerals from biochar, P sorption and organic acid sorption to biochar, the formation of organo-Fe complexes on biochar and interference with plant-fungi signaling pathways. Optimizing P delivery with co-pyrolyzed biochar and P deposition into

biochar warrant further research. Future studies should also focus on the mechanisms by which biochar affects AM colonization and the efficacy of bone char as a P fertilizer in other soil environments

Supporting Information

Supporting data associated with this article follows.

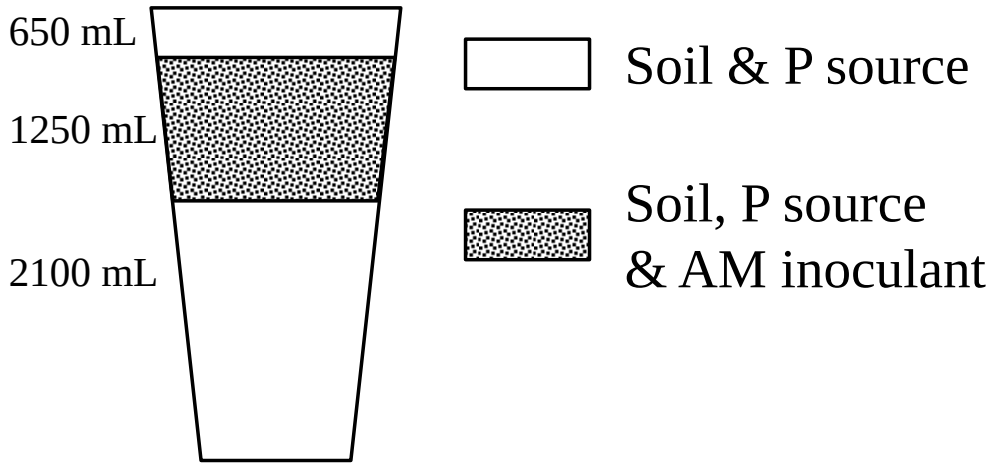

Figure 2.S1: AM inoculant application in soil

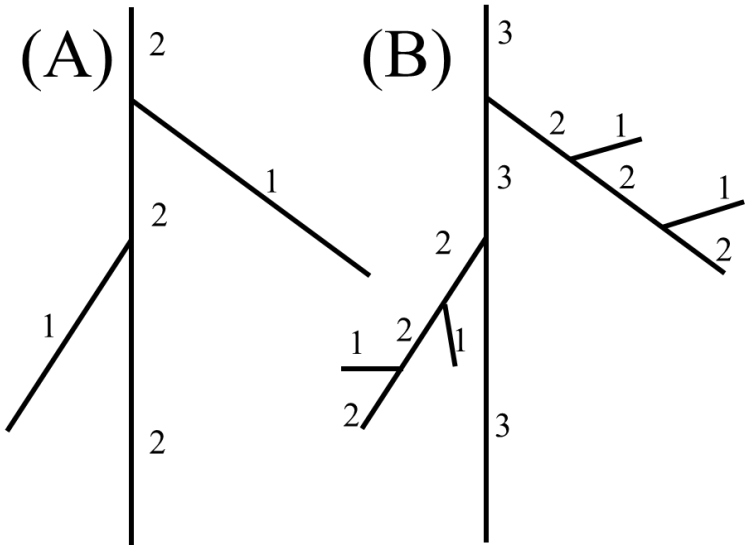

Figure 2.S2: Naming scheme of link analysis for root systems with (A) two root orders and (B) three orders**.**

Table 2.S1: Total Phosphorus (Total-P) content, total calcium (Total-Ca) content and molar Ca/P ratio of P sources (means and standard deviation, n=3): rendered bone char produced at 750°C (RB750), rendered bone-wood char produced at 750°C (RBW750) and triple super phosphate (TSP) fertilizer

P source	Total P (mg g^{-1})	Total Ca (mg g^{-1})	Molar Ca/P ratio
RB750	153.24±2.17	337.07±8.25	1.70±0.05
RBW750	116.55±2.59	258.29±4.50	1.71±0.02
TSP	205.25±1.85	161.02±5.25	0.61±0.02

References

Abelson, P. H. 1999. A potential phosphate crisis. *Science* 283 (5410).

Akiyama, K., K. Matsuzaki, and H. Hayashi. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435 (7043).

Antelo, J., F. Arce, M. Avena, S. Fiol, R. Lopez, and F. Macias. 2007. Adsorption of a soil humic acid at the surface of goethite and its competitive interaction with phosphate. *Geoderma* 138 (1-2).

Ayllon, M., M. Aznar, J. L. Sanchez, G. Gea, and J. Arauzo. 2006. Influence of temperature and heating rate on the fixed bed pyrolysis of meat and bone meal. *Chemical Engineering Journal* 121 (2-3).

Bates, T. R., and J. P. Lynch. 2001. Root hairs confer a competitive advantage under low phosphorus availability. *Plant and Soil* 236 (2).

Benton Jones, J. 2001. *Laboratory Guide for Conducting Soil Tests and Plant Analysis*. Boca Raton, USA: CRC Press LLC.

Berta, G., A. Fusconi, and A. Trotta. 1993. VA mycorrhizal infection and the morphology and function of root systems. *Environmental and Experimental Botany* 33 (1).

Bolan, N. S., R. Naidu, S. Mahimairaja, and S. Baskaran. 1994. Influence of low molecular wieght organic acids on the solubilitzaion of phosphates. *Biology and Fertility of Soils* 18 (4).

Brown, W. E., P. R. Patel, and L. C. Chow. 1975. Formation of $CaHPO_4$ $2H_2O$ from enamel mineral and it relationship to caries mechanism. *Journal of Dental Research* 54 (3).

Cascarosa, E., G. Gea, and J. Arauzo. 2012. Thermochemical processing of meat and bone meal: A review. *Renewable & Sustainable Energy Reviews* 16 (1).

Cheng, C., J. Lehmann, and M. H. Engelhard. 2008. Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence. *Geochimica Et Cosmochimica Acta* 72 (6).

Cordell, D., J. O. Drangert, and S. White. 2009. The story of phosphorus: Global food security and food for thought. *Global Environmental Change-Human and Policy Dimensions* 19 (2).

Cordell, D., A. Rosemarin, J. J. Schroder, and A. L. Smit. 2011. Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere* 84 (6).

Cornelissen, G., O. Gustafsson, T. D. Bucheli, M. T. O. Jonker, A. A. Koelmans, and P. C. M. Van Noort. 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environmental Science & Technology* 39 (18).

Cui, H. J., M. K. Wang, M. L. Fu, and E. Ci. 2011. Enhancing phosphorus availability in phosphorus-fertilized zones by reducing phosphate adsorbed on ferrihydrite using rice straw-derived biochar. *Journal of Soils and Sediments* 11 (7).

DeLuca, T H, M D MacKanzie, and M J Gundale. 2009. Bio-char effects on soil nutrient transformation. In *Biochar for Environmental Management: Science and Technology*, edited by J. Lehmann, and S. Joseph. London: Earthscan Publications Ltd.

Deydier, E., R. Guilet, S. Sarda, and P. Sharrock. 2005. Physical and chemical characterisation of crude meat and bone meal combustion residue: "waste or raw material?". *Journal of Hazardous Materials* 121 (1-3).

Earl, K. D., J. K. Syers, and J. R. McLaughlin. 1979. Origin of the effects of citrate, tartrate, and acetate on phosphate soprtion by soils and synthetic gels. *Soil Science Society of America Journal* 43 (4).

Fitter, A. 1991. Characteristics and functions of root systems. In *Plant Roots: the Hidden Half*, edited by Y. Waisel, A. Eshel, and U. Kafkafi. New York: Marcel Dekker Inc.

Gerke, J., and R. Hermann. 1992. Adsorption of Orthophosphate to Humic-Fe-Complexes and to Amorphous Fe-Oxide. *Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* 155 (3).

Guppy, C. N., N. W. Menzies, P. W. Moody, and F. P. C. Blamey. 2005. Competitive sorption reactions between phosphorus and organic matter in soil: a review. *Australian Journal of Soil Research* 43 (2).

Haynes, R. J., and M. S. Mokolobate. 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. *Nutrient Cycling in Agroecosystems* 59 (1).

Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil* 237 (2).

Hochholdinger, F., T. Wen, R. Zimmermann, et al. 2008. The maize (Zea mays L.) roothairless3 gene encodes a putative GPI-anchored, monocot-specific, COBRA-like protein that significantly affects grain yield. *Plant Journal* 54 (5).

Hodge, A., G. Berta, C. Doussan, F. Merchan, and M. Crespi. 2009. Plant root growth, architecture and function. *Plant and Soil* 321 (1-2).

Hollister, C. C., J. J. Bisogni, and J. Lehmann. 2013. Ammonium, Nitrate, and Phosphate Sorption to and Solute Leaching from Biochars Prepared from Corn Stover (Zea mays L.) and Oak Wood (Quercus spp.). *J. Environ. Qual.* 42 (1).

Hunt, J. F., T. Ohno, Z. He, C. W. Honeycutt, and D. B. Dail. 2007. Inhibition of phosphorus sorption to goethite, gibbsite, and kaolin by fresh and decomposed organic matter. *Biology and Fertility of Soils* 44 (2).

Jones, D. L. 1998. Organic acids in the rhizosphere - a critical review. *Plant and Soil* 205 (1).

Kaldorf, M., and J. Ludwig-Muller. 2000. AM fungi might affect the root morphology of maize by increasing indole-3-butyric acid biosynthesis. *Physiologia Plantarum* 109 (1).

Kingombe, C. I. B., E. Luthi, H. Schlosser, D. Howald, M. Kuhn, and T. Jemmi. 2001. A PCR-based test for species-specific determination of heat treatment conditions of animal meals as an effective prophylactic method for bovine spongiform encephalopathy. *Meat Science* 57 (1).

Kucey, R. M. N., H. H. Janzen, and M. E. Leggett. 1989. Microbially mediated increases in plant available phosphorus. *Advances in Agronomy* 42.

Kuo, S. 1996. Phosphorus. In *Methods of Soil Analysis - Part 3 Chemical Methods*, edited by D. L. Sparks. Madison, Wisconsin, USA: Soil Science Society of America, Inc. & American Society of Agronomy.

Lehmann, J., J. P. da Silva, C. Steiner, T. Nehls, W. Zech, and B. Glaser. 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil* 249 (2).

Lehmann, J., M. C. Rillig, J. Thies, C. A. Masiello, W. C. Hockaday, and D. Crowley. 2011. Biochar effects on soil biota - A review. *Soil Biology & Biochemistry* 43 (9).

Lynch, J. P. 2011. Root Phenes for Enhanced Soil Exploration and Phosphorus Acquisition: Tools for Future Crops. *Plant Physiology* 156 (3).
Lynch, J. P., and S. E. Beebe. 1995. Adaptation of beans (Phaseolus vulgaris, L) to low phosphorus availability. *Hortscience* 30 (6).
Lynch, J. P. 2007. Roots of the second green revolution. *Australian Journal of Botany* 55 (5).
Marschner, H. 1995. Mineral Nutrition of Higher Plants. London: Academic Press.
Matsumoto, T., M. Okazaki, M. Inoue, Y. Hamada, M. Taira, and J. Takahashi. 2002. Crystallinity and solubility characteristics of hydroxyapatite adsorbed amino acid. *Biomaterials* 23 (10).
Murphy, J., and J. P. Riley. 1962. A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta* 26 (1).
Nelson, N. O., S. C. Agudelo, W. Q. Yuan, and J. Gan. 2011. Nitrogen and Phosphorus Availability in Biochar-Amended Soils. *Soil Science* 176 (5).
Neset, T. S. S., and D. Cordell. 2012. Global phosphorus scarcity: identifying synergies for a sustainable future. *Journal of the Science of Food and Agriculture* 92 (1).
Parfitt, R. L., R. J. Atkinson, and R. S. C. Smart. 1975. Mechanism of phosphate fixation by iron oxides. *Soil Science Society of America Journal* 39 (5).
Raghothama, K. G., and A. S. Karthikeyan. 2005. Phosphate acquisition. *Plant and Soil* 274 (1-2).
Rajan, S. S. S., J. H. Watkinson, and A. G. Sinclair. 1996. Phosphate rocks for direct application to soils. *Advances in Agronomy, Vol 57* 57.
Ramaekers, L., R. Remans, I. M. Rao, M. W. Blair, and J. Vanderleyden. 2010. Strategies for improving phosphorus acquisition efficiency of crop plants. *Field Crops Research* 117 (2-3).
Richardson, Alan E., Jonathan P. Lynch, Peter R. Ryan, et al. 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil* 349 (1-2).
Sanchez, P. A. 1976. Properties and Management of Acid Soils in the Tropics. New York: John Wiley.
Sattelmacher, B., J. Gerendas, K. Thoms, H. Bruck, and N. H. Bagdady. 1993. Interaction between root growth and mineral nutrition. *Environmental and Experimental Botany* 33 (1).
Siebers, N., F. Godlinski, and P. Leinweber. 2012. The phosphorus fertilizer value of bone char for potatoes, wheat and onions: first results. *Landbauforschung Volkenrode* 62 (1/2).
Singh, B. B., and J. P. Jones. 1976. Phosphorus soprtion and desorption characteristics of soil as affected by organic residues. *Soil Science Society of America Journal* 40 (3).
Smil, V. 2000. Phosphorus in the environment: Natural flows and human interferences. *Annual Review of Energy and the Environment* 25.
Smits, M. M., S. Bonneville, L. G. Benning, S. A. Banwart, and J. R. Leake. 2012. Plant-driven weathering of apatite - the role of an ectomycorrhizal fungus. *Geobiology* 10 (5).
Tiessen, H, and JO Moir. 1993. Characterization of available P by sequential fractionation. In *Soil sampling and methods of analysis*, edited by M. Carter. Boca Raton, Florida, USA: Lewis.
Tuominen, L., T. Kairesalo, and H. Hartikainen. 1994. Comparison of methods for inhibiting bacterial activity in sediment. *Applied and Environmental Microbiology* 60 (9).
Vance, C. P., C. Uhde-Stone, and D. L. Allan. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist* 157 (3).

Vierheilig, H. 2004. Regulatory mechanisms during the plant-arbuscular mycorrhizal fungus interaction. *Canadian Journal of Botany-Revue Canadienne De Botanique* 82 (8).
Warnock, D. D., J. Lehmann, T. W. Kuyper, and M. C. Rillig. 2007. Mycorrhizal responses to biochar in soil - concepts and mechanisms. *Plant and Soil* 300 (1-2).
Warren, G. P., J. S. Robinson, and E. Someus. 2009. Dissolution of phosphorus from animal bone char in 12 soils. *Nutrient Cycling in Agroecosystems* 84 (2).
Wen, T. J., and P. S. Schnable. 1994. Analyses of mutants of 3 genes that influence root hair development in Zea mays (Gramineae) suggest that root hairs are dispensable. *American Journal of Botany* 81 (7).
Wopenka, B., and J. D. Pasteris. 2005. A mineralogical perspective on the apatite in bone. *Materials Science & Engineering C-Biomimetic and Supramolecular Systems* 25 (2).
Wopenka, B., and J. Pasteris. 2003. Biological apatites: A comparison of bone and tooth mineralization. *Journal of Vertebrate Paleontology* 23 (3 Supplement).
Zhu, J., C. Zhang, and J. P. Lynch. 2010. The utility of phenotypic plasticity of root hair length for phosphorus acquisition. *Functional Plant Biology* 37 (4).
Zwetsloot, M. J., J. Lehmann, D. Solomon. Unpublished. Recycling slaughterhouse waste into fertilizer: how do pyrolysis temperature and biomass additions affect phosphorus chemistry in bone char?

APPENDIX 1

PRIMARY DATA FOR CHAPTER ONE

Table A1.1: pH of different feedstock dried or charred at temperature (T) 60, 220, 350, 550 or 750°C and commercial fertilizers: bone and meat residue (BM), bone with meat residue and corn biomass (BC), bone with meat residue and wood biomass (BW), rendered bone (RB), rendered bone with wood biomass (RBW), corn biomass (corn), wood biomass (wood), Idaho rock phosphate (rock P) and triple superphosphate (TSP). Measurements were taken in duplicates.

Feedstock	**T (°C)**	**pH 1**	**pH 2**
BM	60	6.26	6.24
BM	220	6.7	6.69
BM	350	7.96	9.12
BM	550	10.6	10.62
BM	750	10.97	10.96
BC	220	6.7	6.73
BC	350	9.61	9.5
BC	550	10.26	10.27
BC	750	10.36	10.36
BW	220	6.47	6.46
BW	350	8.2	8.31
BW	360	8.16	8.23
BW	550	10.16	10.08
BW	750	10.51	10.5
RB	60	7.34	7.35
RB	220	6.8	6.79
RB	350	7.48	7.48
RB	550	9.23	9.15
RB	750	10.24	10.15
RBW	220	6.64	6.65
RBW	350	7.27	7.19
RBW	550	8.75	8.81
RBW	750	10.09	10.07
Corn	60	7.22	7.16
Corn	220	7.05	7.04
Corn	350	10.09	10.07
Corn	550	10.15	10.2
Corn	750	10.4	10.37
Wood	60	5.27	5.19
Wood	220	5.01	4.89

Table A1.1 (Continued)

Wood	350	7.08	6.99
Wood	550	9.81	9.84
Wood	750	10.2	10.24

Table A1.2: Formic acid extractable phosphorus (Formic-P) measurements of different feedstock dried or charred at temperature (T) 60, 220, 350, 550 or 750°C and commercial fertilizers: bone and meat residue (BM), bone with meat residue and corn biomass (BC), bone with meat residue and wood biomass (BW), rendered bone (RB), rendered bone with wood biomass (RBW), corn biomass (corn), Idaho rock phosphate (rock P) and triple superphosphate (TSP). Measurements were taken in triplicates.

Feedstock	T (°C)	Formic-P (mg g^{-1} output)		
		Replicate 1	Replicate 2	Replicate 3
BM	60	31.25	25.00	27.94
BM	220	18.06	11.81	15.24
BM	350	64.24	70.14	68.21
BM	550	88.89	89.72	90.70
BM	750	95.83	98.98	99.41
BC	220	13.54	14.24	11.79
BC	350	30.56	30.80	32.65
BC	550	50.35	49.84	50.43
BC	750	50.00	54.12	55.15
BW	220	13.54	15.97	14.51
BW	350	30.21	28.66	32.83
BW	550	42.48	48.06	46.44
BW	750	51.39	55.54	58.41
Corn	60	0.30	0.28	0.28
Corn	220	0.42	0.30	0.32
Corn	350	0.80	0.86	0.91
Corn	550	0.76	0.70	0.58
Corn	750	1.18	1.01	0.93
RB	60	77.08	78.47	81.89
RB	220	80.56	86.11	88.29
RB	350	113.89	127.46	117.49
RB	550	136.81	139.56	138.14
RB	750	144.86	143.84	152.38
RBW	220	47.22	48.42	47.89
RBW	350	100.70	99.69	105.94
RBW	550	100.00	100.40	105.21
RBW	750	113.89	118.91	124.08
Wood	60	0.04	0.05	0.04
Wood	220	0.04	0.05	0.05
Wood	350	0.06	0.05	0.05
Wood	550	0.14	0.13	0.10
Wood	750	0.32	0.31	0.31
Rock P	n/a	23.26	23.50	24.31
TSP	n/a	195.14	189.41	197.36

Table A1.3: Water-extractable phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) of different feedstock dried or charred at temperature (T) 60, 220, 350, 550 or 750°C and commercial fertilizers: bone and meat residue (BM), bone with meat residue and corn biomass (BC), bone with meat residue and wood biomass (BW), rendered bone (RB), rendered bone with wood biomass (RBW), corn biomass (corn), Idaho rock phosphate (rock P) and triple superphosphate (TSP). Measurements were taken in triplicates.

Feedstock	T (°C)	Water-soluble elements (mg g^{-1} output)				
		P	Ca	Mg	Na	K
BM	60	5.58	4.78	0.74	1.65	6.59
BM	60	5.08	4.13	0.69	1.56	6.17
BM	60	4.63	3.60	0.65	1.44	5.77
BM	220	3.09	1.41	0.33	1.43	6.68
BM	220	3.41	1.51	0.39	1.55	7.15
BM	220	3.07	1.27	0.32	1.55	7.18
BM	350	1.02	0.44	0.15	0.31	1.58
BM	350	0.92	0.38	0.14	0.30	1.55
BM	350	1.01	0.46	0.13	0.30	1.54
BM	550	2.90	0.67	0.35	1.36	9.11
BM	550	3.05	0.77	0.38	1.38	9.16
BM	550	2.97	0.70	0.35	1.33	8.74
BM	750	3.16	0.62	0.20	2.08	12.43
BM	750	3.25	0.66	0.21	2.12	12.50
BM	750	3.32	0.68	0.21	2.13	12.61
BC	220	3.58	2.11	0.88	1.10	10.40
BC	220	3.45	2.13	0.86	1.07	9.75
BC	220	3.29	2.14	0.88	1.07	9.76
BC	350	0.66	0.35	0.19	0.37	5.63
BC	350	0.66	0.37	0.18	0.39	5.51
BC	350	0.70	0.40	0.18	0.37	5.45
BC	550	2.39	0.21	0.10	1.22	13.45
BC	550	2.37	0.24	0.10	1.19	12.59
BC	550	2.60	0.35	0.11	1.18	12.12
BC	750	2.37	0.30	0.10	1.13	11.56
BC	750	2.54	0.37	0.10	1.21	12.09
BC	750	2.55	0.43	0.10	1.19	12.14
BW	220	3.30	2.19	0.42	0.85	4.11
BW	220	3.14	2.09	0.41	0.84	3.94
BW	220	2.98	2.08	0.40	0.81	3.80
BW	350	0.56	0.46	0.11	0.25	1.36
BW	350	0.57	0.49	0.11	0.27	1.52
BW	350	0.65	0.50	0.11	0.28	1.48
BW	550	1.81	0.29	0.14	0.86	5.23

Table A1.3 (Continued)

BW	550	1.88	0.32	0.14	0.92	5.50
BW	550	2.12	0.45	0.15	0.93	5.45
BW	750	1.42	0.34	0.06	0.89	5.26
BW	750	1.64	0.45	0.07	0.96	5.55
BW	750	1.56	0.47	0.08	0.93	5.45
RB	60	1.64	2.89	0.66	1.97	1.36
RB	60	1.88	4.02	0.72	2.00	1.16
RB	60	1.57	2.10	0.65	1.86	1.72
RB	220	1.79	2.23	0.88	2.11	1.92
RB	220	1.65	2.20	0.85	1.86	1.80
RB	220	1.70	2.22	0.84	1.87	1.75
RB	350	0.41	2.34	0.81	1.34	1.47
RB	350	0.43	2.36	0.81	1.36	1.54
RB	350	0.45	2.21	0.73	1.30	1.46
RB	550	0.70	0.74	0.24	1.87	1.91
RB	550	0.66	0.72	0.22	1.93	1.96
RB	550	0.60	0.48	0.18	1.90	1.89
RB	750	0.56	0.81	0.03	2.46	1.78
RB	750	0.49	0.67	0.03	2.46	1.92
RB	750	0.51	0.68	0.03	2.47	1.95
RBW	220	1.65	1.98	0.52	0.93	1.08
RBW	220	1.61	1.96	0.54	0.93	1.15
RBW	220	1.60	1.97	0.55	0.96	1.23
RBW	350	0.65	2.12	0.57	0.76	1.06
RBW	350	0.72	2.14	0.53	0.80	1.00
RBW	350	0.60	1.94	0.57	0.79	1.11
RBW	550	0.50	0.43	0.21	1.36	1.34
RBW	550	0.48	0.42	0.22	1.48	1.55
RBW	550	0.49	0.37	0.21	1.49	1.60
RBW	750	0.40	0.29	0.03	1.95	1.53
RBW	750	0.42	0.30	0.03	2.02	1.68
RBW	750	0.46	0.38	0.03	2.07	1.73
Corn	60	0.31	0.73	0.76	0.08	8.77
Corn	60	0.31	0.72	0.73	0.07	8.13
Corn	60	0.30	0.71	0.74	0.07	8.41
Corn	220	0.51	0.59	0.60	0.09	11.72
Corn	220	0.52	0.66	0.62	0.09	11.94
Corn	220	0.50	0.59	0.59	0.10	11.91
Corn	350	0.54	0.22	0.35	0.11	14.26
Corn	350	0.58	0.27	0.35	0.13	14.01
Corn	350	0.57	0.26	0.34	0.12	13.79

Table A1.3 (Continued)

Corn	550	0.32	0.26	0.29	0.10	22.54
Corn	550	0.32	0.27	0.28	0.11	22.12
Corn	550	0.36	0.27	0.28	0.12	23.07
Corn	750	0.21	0.95	0.55	0.24	25.38
Corn	750	0.22	0.92	0.54	0.25	25.18
Corn	750	0.22	0.90	0.54	0.27	25.64
Rock P		0.40	12.80	0.57	1.87	4.02
Rock P		0.31	13.36	0.55	1.75	5.23
Rock P		0.26	13.49	0.55	1.78	5.49
TSP		329.36	148.81	6.35	4.33	7.55
TSP		236.06	144.56	6.46	3.65	1.49
TSP		216.02	140.54	6.21	3.58	1.71

Table A1.4: Total elemental content of phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), sulfur (S), iron (Fe) and aluminum (Al) of different feedstock dried or charred at temperatures (T) 60, 220, 350, 550 or 750°C and commercial fertilizers: bone and meat residue (BM), bone with meat residue and corn biomass (BC), bone with meat residue and wood biomass (BW), rendered bone (RB), rendered bone with wood biomass (RBW), corn biomass (corn), wood biomass (wood), Idaho rock phosphate (rock P) and triple superphosphate (TSP). Measurements were taken in triplicates.

Feedstock	T (°C)	Total Elemental Content (mg g output^{-1})							
		P	Ca	Mg	K	Na	S	Fe	Al
BM	60	29.55	52.85	1.70	6.56	1.75	1.45	0.13	0.11
BM	60	36.50	66.27	1.78	6.21	1.83	1.17	0.10	0.13
BM	60	31.72	57.04	1.65	5.77	1.78	1.02	0.10	0.09
BM	220	30.81	58.11	1.69	6.93	2.01	1.09	0.11	0.11
BM	220	29.96	53.65	1.63	6.45	1.92	1.51	0.13	0.12
BM	220	33.13	60.00	1.76	6.16	2.03	0.91	0.15	0.10
BM	350	87.45	159.15	4.21	15.53	4.73	2.05	0.27	0.18
BM	350	81.99	148.92	4.01	15.26	4.65	2.11	0.29	0.21
BM	350	80.70	147.04	4.04	15.15	4.95	2.01	0.26	0.17
BM	550	105.67	197.33	5.34	20.49	6.27	0.94	0.62	0.16
BM	550	103.53	193.98	5.19	19.82	6.06	0.86	0.57	0.21
BM	550	103.11	190.88	5.07	19.91	6.01	0.85	0.59	0.18
BM	750	109.41	199.42	5.59	23.01	7.12	1.29	1.36	0.22
BM	750	109.33	202.52	5.45	22.15	6.95	1.26	1.49	0.18
BM	750	109.88	210.40	5.53	22.48	6.57	1.22	1.18	0.17
BC	220	15.08	27.00	1.75	8.82	1.07	1.47	0.53	0.48
BC	220	14.10	24.99	1.77	8.44	1.09	4.78	0.61	0.52
BC	220	13.85	24.14	1.62	7.78	1.27	1.34	0.51	0.48
BC	350	38.82	70.95	3.59	16.01	2.37	1.53	0.86	0.85
BC	350	38.64	71.40	3.47	15.03	2.42	1.41	0.91	0.89
BC	350	35.94	67.05	3.41	15.05	2.56	1.39	0.82	0.86
BC	550	56.31	101.57	4.71	22.64	3.77	1.36	1.03	1.23
BC	550	57.09	102.02	4.57	22.02	3.88	1.28	0.96	1.29
BC	550	53.81	98.29	4.61	22.61	3.59	1.28	0.98	1.09
BC	750	57.62	107.31	4.79	23.01	4.23	1.27	1.15	1.26
BC	750	59.28	110.20	4.67	22.01	4.19	1.23	1.11	1.23
BC	750	57.45	112.66	4.69	22.09	3.74	1.18	1.20	1.17
BW	220	15.64	28.91	0.92	3.98	0.93	0.79	0.07	0.10
BW	220	14.46	26.11	0.86	3.54	0.88	0.80	0.09	0.19
BW	220	16.18	28.39	0.88	3.45	0.96	0.80	0.07	0.11
BW	350	40.60	74.48	2.22	9.17	2.37	1.44	0.16	0.15
BW	350	39.84	72.78	2.18	8.73	2.33	1.41	0.18	0.13
BW	350	38.67	69.16	2.15	8.54	2.48	1.42	0.17	0.10

Table A1.4 (Continued)

BW	550	55.47	99.01	3.03	12.63	3.35	1.17	0.25	0.17
BW	550	53.26	95.55	2.85	11.92	3.22	0.99	0.26	0.21
BW	550	50.47	91.63	2.75	11.83	3.17	0.97	0.27	0.18
BW	750	54.63	100.51	3.22	13.66	3.83	0.96	0.56	0.16
BW	750	57.43	108.55	3.27	13.33	3.80	0.97	0.71	0.19
BW	750	57.17	109.83	3.29	13.35	3.70	0.94	0.72	0.16
RB	60	87.34	187.78	3.57	2.08	4.24	1.34	0.31	0.18
RB	60	89.61	191.05	3.78	2.25	4.33	1.46	0.24	0.18
RB	60	80.65	171.49	3.34	1.66	4.33	1.21	0.19	0.15
RB	220	87.90	196.26	3.72	2.46	4.48	1.39	0.24	0.17
RB	220	88.39	186.78	3.67	1.79	4.64	3.07	0.24	0.18
RB	220	87.49	185.14	3.57	1.86	4.59	1.38	0.17	0.18
RB	350	128.32	274.94	5.35	2.74	5.90	1.59	0.13	0.20
RB	350	127.27	274.54	5.18	2.46	5.72	1.60	0.15	0.22
RB	350	125.70	262.21	5.07	2.22	6.01	1.51	0.16	0.20
RB	550	144.27	310.83	5.94	3.15	6.55	1.60	0.15	0.20
RB	550	139.99	309.81	5.67	2.71	6.38	1.42	0.27	0.21
RB	550	135.38	301.04	5.68	2.58	6.44	1.39	0.16	0.20
RB	750	153.16	327.68	6.04	3.43	7.47	1.01	0.93	0.28
RB	750	155.44	340.43	5.93	2.89	7.33	0.97	0.92	0.22
RB	750	151.10	343.12	5.87	2.68	7.10	0.99	0.99	0.20
RBW	220	48.31	106.96	2.07	1.88	2.25	0.70	0.39	0.13
RBW	220	48.24	102.80	2.04	1.46	2.28	0.74	0.37	0.14
RBW	220	45.52	96.47	1.91	1.12	2.19	0.56	0.48	0.14
RBW	350	103.76	226.60	4.53	2.67	4.62	1.12	0.13	0.19
RBW	350	103.06	227.09	4.46	2.25	4.58	1.01	0.13	0.28
RBW	350	100.49	219.61	4.39	1.98	4.78	1.00	0.11	0.20
RBW	550	100.71	219.00	4.34	3.55	4.66	1.07	0.15	0.18
RBW	550	104.79	222.13	4.38	2.42	4.97	1.12	0.15	0.17
RBW	550	97.17	214.84	4.18	2.41	4.56	1.01	0.11	0.17
RBW	750	114.07	255.65	4.87	3.21	5.67	0.65	0.29	0.24
RBW	750	119.24	263.48	4.71	2.76	5.46	0.67	0.23	0.22
RBW	750	116.35	255.72	4.58	2.12	5.51	0.61	0.32	0.16
Corn	60	0.60	2.28	1.77	9.17	0.13	0.53	0.43	0.39
Corn	60	0.56	2.16	1.71	9.19	0.14	0.52	0.41	0.34
Corn	60	0.55	2.08	1.67	8.49	0.35	0.48	0.38	0.39
Corn	220	0.60	2.44	1.60	12.14	0.23	0.61	0.62	0.59
Corn	220	0.58	2.42	1.59	12.46	0.16	0.57	1.34	0.64
Corn	220	0.62	2.32	1.60	12.32	0.57	0.59	0.63	0.64
Corn	350	1.35	5.78	3.91	24.40	0.73	0.90	1.48	1.41
Corn	350	1.43	5.71	4.00	25.93	0.73	0.94	1.67	1.48

Table A1.4 (Continued)

Corn	350	1.41	5.49	3.93	25.95	0.66	0.92	1.60	1.49
Corn	550	1.55	6.19	5.09	30.86	1.06	0.89	2.07	2.25
Corn	550	1.52	6.22	5.07	30.25	0.84	0.86	2.03	2.10
Corn	550	1.47	5.87	4.93	30.69	1.17	0.83	2.05	1.98
Corn	750	1.89	7.12	5.55	31.11	1.41	0.94	1.89	2.28
Corn	750	1.86	7.10	5.57	31.33	1.15	0.95	1.94	2.29
Corn	750	1.82	7.37	5.54	30.42	1.01	0.91	1.64	2.15
Wood	60	0.08	1.11	0.18	0.51	0.10	0.05	0.04	0.03
Wood	60	0.05	1.08	0.18	0.52	0.09	0.06	0.02	0.03
Wood	60	0.04	1.04	0.16	0.47	0.09	0.04	0.02	0.02
Wood	220	0.10	1.17	0.19	0.58	0.12	0.06	0.04	0.08
Wood	220	0.10	1.09	0.18	0.55	0.09	0.06	0.03	0.08
Wood	220	0.06	1.03	0.17	0.55	0.08	0.05	0.02	0.03
Wood	350	0.11	3.04	0.46	1.50	0.17	0.09	0.08	0.09
Wood	350	0.14	2.86	0.43	1.49	0.19	0.08	0.11	0.09
Wood	350	0.16	2.80	0.43	1.38	0.24	0.09	0.10	0.07
Wood	550	0.22	4.34	0.60	2.38	0.24	0.11	0.05	0.11
Wood	550	0.21	4.09	0.57	2.19	0.28	0.09	0.06	0.17
Wood	550	0.13	3.83	0.55	2.18	0.31	0.09	0.02	0.04
Wood	750	0.33	4.85	0.67	2.36	0.49	0.17	0.16	0.06
Wood	750	0.15	4.74	0.64	2.43	0.39	0.16	0.02	0.06
Wood	750	0.37	4.72	0.63	2.57	0.32	0.16	0.14	0.09
Rock P		86.48	228.51	2.64	8.45	2.65	15.66	8.40	10.02
Rock P		83.96	220.11	2.70	9.69	2.83	16.17	8.32	11.30
Rock P		84.11	223.38	2.75	9.61	2.73	15.75	8.50	11.48
TSP		205.11	166.63	6.01	1.46	3.56	12.36	1.26	0.94
TSP		203.48	156.24	6.10	1.76	3.89	10.10	1.27	1.04
TSP		207.17	160.19	6.00	1.24	3.90	9.96	1.26	1.01

APPENDIX 2

PRIMARY DATA FOR CHAPTER TWO

Table A2.1: Phosphorus (P) adsorption isotherm of soil from research farm of Jimma University in Ethiopia that was used for abiotic incubation and pot trial.

P in solution (mg L^{-1})	**P adsorbed (mg kg $soil^{-1}$)**
0	0
0	10
0	20
0	50
1	88
3	169
18	317
40	403
57	433

Table A2.2: Soil water retention of soil from research farm of Jimma University in Ethiopia that was used for abiotic incubation and pot trial.

Pressure (cm H_2O)	Gravimetric water content
0.01 (saturated)	0.79
0.01 (saturated)	0.75
0.01 (saturated)	0.78
0.01 (saturated)	0.85
10	0.60
10	0.59
10	0.81
10	0.80
20	0.59
20	0.61
20	0.57
20	0.58
50	0.44
50	0.44
50	0.45
50	0.43
100	0.41
100	0.41
100	0.39
100	0.39
200	0.40
200	
200	0.37
200	0.37
1000	0.33
1000	0.33
1000	0.27
1000	0.27

Table A2.3: pH measurements in deionized water and 1 M KCl solution of soil from research farm of Jimma University in Ethiopia that was used for abiotic incubation and pot trial. Measurements were taken in quadruples.

Replicate	water-pH	KCl-pH
1	4.74	4.04
2	4.45	4.05
3	4.87	4.10
4	4.2	3.75

Table A2.4: Total carbon (C) and total nitrogen (N) in of soil from research farm of Jimma University in Ethiopia that was used for abiotic incubation and pot trial

Replicate	%N	%C
1	0.27	3.05
2	0.27	3.06

Table A2.5: Cation-exchange capacity potential of soil from research farm of Jimma University in Ethiopia that was used for abiotic incubation and pot trial

Replicate	Cation-exchange capacity (mmol kg^{-1} soil)
1	275.42
2	278.43
3	280.75
4	281.97

Table A2.6: Exchangeable base cations calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) of soil from research farm of Jimma University in Ethiopia that was used for abiotic incubation and pot trial.

Replicate	Ca (g kg soil^{-1})	K (g kg soil^{-1})	Mg (g kg soil^{-1})	Na (g kg soil^{-1})
1	5.33	3.12	1.23	0.05
2	5.21	2.65	1.19	0.08
3	5.12	2.93	1.17	0.05

Table A2.7: Mehlich-III extractable phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) of soil from research farm of Jimma University in Ethiopia that was used for abiotic incubation and pot trial.

Replicate	**P (mg kg soil^{-1})**	**Ca (mg kg soil^{-1})**	**K (mg kg soil^{-1})**	**Mg (mg kg soil^{-1})**	**Zn (mg kg soil^{-1})**	**Fe (mg kg soil^{-1})**	**Mn (mg kg soil^{-1})**	**Cu (mg kg soil^{-1})**
1	0.00	682.63	356.63	135.26	4.76	65.53	163.53	0.39
2	0.00	688.32	344.68	97.13	4.01	72.94	167.30	0.31

Table A2.8: Texture classification of soil around Jimma in Ethiopia that was used for abiotic incubation and pot trial. Measurements were taken in duplicate.

Soil	clay (%)	silt (%)	sand (%)
Replicate 1	48	44	8
Replicate 2	52	36	12

Table A2.9: Resin- and Olsen-extractable P from an acid, P-fixing soil incubated with commercial fertilizer and bone fertilizers dried or charred at 350 or 750°C in the presence or absence of wood biochar. Feedstock include: rendered bone (RB), separate additions of rendered bone and wood biomass (RB + W), co-pyrolyzed rendered bone with wood biomass (RBW), triple superphosphate (TSP) fertilizer, separate additions of TSP and wood biochar (TSP + W), TSP placed in wood biochar pores (TSPW).

Feedstock	T (°C)	Resin-P (mg kg^{-1} soil)	Olsen-P (mg kg^{-1} soil)
RB	60	38.7	45.6
RB	60	51.0	47.0
RB	60	26.3	41.5
RB	60	54.7	43.2
RB	60	33.5	46.6
RB	350	57.3	40.7
RB	350	73.5	44.5
RB	350	68.7	32.0
RB	350	82.3	39.9
RB	350	60.4	42.7
RB	750	35.2	34.1
RB	750	41.2	33.1
RB	750	53.5	32.5
RB	750	46.5	32.0
RB	750	43.0	34.2
RB + W	60	50.7	40.1
RB + W	60	52.3	47.2
RB + W	60	51.5	40.8
RB + W	60	45.6	45.4
RB + W	60	51.0	44.2
RB + W	350	62.1	42.5
RB + W	350	31.9	38.6
RB + W	350	62.2	34.3
RB + W	350	60.2	42.3
RB + W	350	50.6	37.8
RB + W	750	36.4	36.8
RB + W	750	34.0	34.3
RB + W	750	37.6	35.3
RB + W	750	22.1	31.8
RB + W	750	33.9	37.3
RBW	350	26.1	42.2
RBW	350	60.2	48.9
RBW	350	58.3	45.1
RBW	350	59.3	57.3

Table A2.9 (Continued)

RBW	350	49.6	44.8
RBW	750	26.6	37.7
RBW	750	39.0	37.2
RBW	750	40.8	39.0
RBW	750	45.3	39.4
RBW	750	37.0	34.5
TSP	n/a	46.0	26.3
TSP	n/a	62.5	36.0
TSP	n/a	61.2	39.9
TSP	n/a	64.9	36.3
TSP	n/a	59.3	37.0
TSP + W	350	57.0	32.4
TSP + W	350	57.2	36.6
TSP + W	350	47.5	34.6
TSP + W	350	60.5	34.9
TSP + W	350	48.1	34.4
TSP + W	750	57.5	34.6
TSP + W	750	60.1	37.6
TSP + W	750	56.8	39.9
TSP + W	750	56.2	36.0
TSP + W	750	56.7	38.4
TSPW	350	40.0	26.8
TSPW	350	31.0	20.5
TSPW	350	32.9	18.7
TSPW	350	34.5	21.6
TSPW	350	33.8	24.1
TSPW	750	39.9	24.3
TSPW	750	42.5	27.7
TSPW	750	44.9	28.2
TSPW	750	45.5	29.3
TSPW	750	42.1	26.5

Table A2.10: Total dry biomass, shoot dry biomass, root dry biomass and root:shoot ratio of maize receiving no additions (NP), rendered bone char produced at 750°C (RB750), co-pyrolyzed rendered bone-wood char produced at 750°C (RBW750) or triple superphosphate (TSP) fertilizer. Soil exploration capacity of maize is altered by maize with no root hairs (NRH), maize with root hairs (RH) and maize with root hairs and inoculated with arbuscular mycorrhizae (RH + AM).

P source	Soil exploration capacity	Total dry biomass (g)	Shoot dry biomass (g)	Root dry biomass (g)	Root:shoot ratio
NP	NRH	0.56	0.35	0.22	0.62
NP	NRH	0.41	0.27	0.14	0.53
NP	NRH	0.50	0.31	0.19	0.62
NP	RH	0.78	0.52	0.26	0.49
NP	RH	0.61	0.43	0.18	0.41
NP	RH	0.62	0.45	0.17	0.39
NP	RH + AM	1.44	0.88	0.56	0.63
NP	RH + AM	0.97	0.61	0.36	0.59
NP	RH + AM	1.07	0.66	0.41	0.63
NP	RH + AM	1.10	0.67	0.43	0.64
NP	RH + AM	1.43	0.93	0.50	0.53
RB750	NRH	0.68	0.42	0.26	0.63
RB750	NRH	0.44	0.24	0.20	0.87
RB750	RH	1.22	0.71	0.51	0.72
RB750	RH	1.73	1.17	0.56	0.48
RB750	RH	1.28	0.82	0.46	0.56
RB750	RH	1.49	1.01	0.48	0.47
RB750	RH	1.37	0.87	0.50	0.58
RB750	RH + AM	2.45	1.38	1.08	0.78
RB750	RH + AM	5.33	2.90	2.42	0.83
RB750	RH + AM	2.28	1.41	0.87	0.61
RB750	RH + AM	3.94	2.23	1.71	0.77
RB750	RH + AM	3.56	2.08	1.48	0.71
RBW750	NRH	0.52	0.32	0.20	0.63
RBW750	NRH	0.74	0.39	0.35	0.90
RBW750	RH	1.34	0.87	0.47	0.53
RBW750	RH	1.25	0.79	0.46	0.58
RBW750	RH	0.91	0.57	0.34	0.60
RBW750	RH	1.70	1.01	0.68	0.67
RBW750	RH + AM	3.51	2.25	1.25	0.56
RBW750	RH + AM	2.55	1.53	1.02	0.67
RBW750	RH + AM	0.89	0.51	0.38	0.74
RBW750	RH + AM	2.79	1.56	1.23	0.79
RBW750	RH + AM	2.36	1.39	0.97	0.70

Table A2.10 (Continued)

TSP	RH	2.66	1.73	0.93	0.54
TSP	RH	2.65	1.80	0.85	0.47
TSP	RH	1.41	0.86	0.55	0.64
TSP	RH	3.29	2.00	1.28	0.64
TSP	RH	2.08	1.25	0.83	0.66
TSP	RH + AM	4.17	2.74	1.44	0.53
TSP	RH + AM	4.69	2.93	1.77	0.60
TSP	RH + AM	5.47	3.41	2.06	0.60
TSP	RH + AM	4.67	2.90	1.77	0.61
TSP	RH + AM	5.44	3.28	2.15	0.65

Table A2.11: Total P accumulation and Ca accumulation of maize receiving no additions (NP), rendered bone char produced at 750°C (RB750), co-pyrolyzed rendered bone-wood char produced at 750°C (RBW750) or triple superphosphate (TSP) fertilizer. Soil exploration capacity of maize altered by maize with no root hairs (NRH), maize with root hairs (RH) and maize with root hairs and inoculated with arbuscular mycorrhizae (RH + AM).

P source	Soil Exploration Capacity	P accumulation (mg plant^{-1})	Ca accumulation (mg plant^{-1})
NP	NRH	0.64	4.27
NP	NRH	0.43	3.39
NP	NRH	0.60	3.79
NP	RH	0.77	6.41
NP	RH	0.70	4.65
NP	RH	0.66	4.48
NP	RH + AM	1.69	8.72
NP	RH + AM	1.15	4.95
NP	RH + AM	1.32	6.67
NP	RH + AM	1.31	6.68
NP	RH + AM	1.79	7.83
RB750	NRH	0.91	5.76
RB750	NRH	0.52	3.04
RB750	RH	1.42	6.62
RB750	RH	2.14	9.10
RB750	RH	1.57	7.37
RB750	RH	1.63	7.07
RB750	RH	1.36	6.97
RB750	RH + AM	4.99	15.24
RB750	RH + AM	8.29	25.64
RB750	RH + AM	3.30	11.51
RB750	RH + AM	7.79	21.48
RB750	RH + AM	5.70	16.21
RBW750	NRH	1.50	6.02
RBW750	NRH	0.75	4.29
RBW750	RH	1.47	7.85
RBW750	RH	1.52	8.03
RBW750	RH	1.00	5.20
RBW750	RH	1.89	9.61
RBW750	RH	5.56	15.14
RBW750	RH + AM	4.12	12.44
RBW750	RH + AM	1.26	4.84
RBW750	RH + AM	5.33	13.65
RBW750	RH + AM	3.86	10.55
TSP	RH	3.60	15.15

Table A2.11 (Continued)

TSP	RH	3.35	13.55
TSP	RH	1.80	7.66
TSP	RH	3.64	14.32
TSP	RH	2.42	10.76
TSP	RH + AM	11.83	17.45
TSP	RH + AM	7.98	20.38
TSP	RH + AM	7.22	27.79
TSP	RH + AM	5.13	18.27
TSP	RH + AM	8.42	22.41

Table A2.12: P concentration and Ca concentration in maize plant tissue receiving no additions (NP), rendered bone char produced at 750°C (RB750), co-pyrolyzed rendered bone-wood char produced at 750°C (RBW750) or triple superphosphate (TSP) fertilizer. Soil exploration capacity of maize altered by maize with no root hairs (NRH), maize with root hairs (RH) and maize with root hairs and inoculated with arbuscular mycorrhizae (RH + AM).

P source	Soil exploration capacity	P concentration (mg g^{-1}plant tissue)	Ca concentration (mg g^{-1} plant tissue)
NP	NRH	1.13	7.61
NP	NRH	1.06	8.34
NP	NRH	1.20	7.58
NP	RH	0.99	8.21
NP	RH	1.16	7.69
NP	RH	1.07	7.26
NP	RH + AM	1.17	6.07
NP	RH + AM	1.19	5.10
NP	RH + AM	1.24	6.25
NP	RH + AM	1.19	6.09
NP	RH + AM	1.25	5.49
RB750	NRH	1.34	8.45
RB750	NRH	1.04	6.09
RB750	RH	1.16	5.43
RB750	RH	1.24	5.26
RB750	RH	1.23	5.78
RB750	RH	1.09	4.75
RB750	RH	0.99	5.09
RB750	RH + AM	2.03	6.21
RB750	RH + AM	1.56	4.81
RB750	RH + AM	1.45	5.05
RB750	RH + AM	1.98	5.45
RB750	RH + AM	1.60	4.55
RBW750	NRH	2.88	11.58
RBW750	NRH	1.00	5.76
RBW750	RH	1.10	5.88
RBW750	RH	1.21	6.44
RBW750	RH	1.09	5.70
RBW750	RH	1.11	5.66
RBW750	RH	1.59	4.32
RBW750	RH + AM	1.62	4.88
RBW750	RH + AM	1.42	5.47
RBW750	RH + AM	1.91	4.90
RBW750	RH + AM	1.63	4.46
TSP	RH	1.36	5.70

Table A2.12 (Continued)

TSP	RH	1.26	5.11
TSP	RH	1.27	5.42
TSP	RH	1.11	4.36
TSP	RH	1.17	5.18
TSP	RH + AM	2.84	4.18
TSP	RH + AM	1.70	4.34
TSP	RH + AM	1.32	5.09
TSP	RH + AM	1.10	3.92
TSP	RH + AM	1.55	4.12

Table A2.13: P accumulation in shoot and root biomass of maize receiving no additions (NP), rendered bone char produced at 750°C (RB750), co-pyrolyzed rendered bone-wood char produced at 750°C (RBW750) or triple superphosphate (TSP) fertilizer. Soil exploration capacity of maize is altered by maize with no root hairs (NRH), maize with root hairs (RH) and maize with root hairs and inoculated with arbuscular mycorrhizae (RH + AM).

P source	Soil exploration capacity	Shoot P accumulation (mg shoot^{-1})	Root P accumulation (mg root^{-1})	Root:shoot P accumulation ratio
NP	NRH	0.41	0.23	0.55
NP	NRH	0.29	0.14	0.49
NP	NRH	0.40	0.20	0.51
NP	RH	0.51	0.27	0.53
NP	RH	0.51	0.19	0.37
NP	RH	0.50	0.16	0.33
NP	RH + AM	1.20	0.49	0.41
NP	RH + AM	0.79	0.37	0.46
NP	RH + AM	0.83	0.49	0.60
NP	RH + AM	0.94	0.37	0.39
NP	RH + AM	1.18	0.61	0.52
RB750	NRH	0.56	0.35	0.63
RB750	NRH	0.31	0.21	0.68
RB750	RH	0.93	0.49	0.52
RB750	RH	1.60	0.54	0.34
RB750	RH	1.02	0.55	0.54
RB750	RH	1.35	0.28	0.21
RB750	RH	1.06	0.30	0.29
RB750	RH + AM	3.41	1.58	0.46
RB750	RH + AM	5.28	3.01	0.57
RB750	RH + AM	2.00	1.31	0.65
RB750	RH + AM	4.48	3.31	0.74
RB750	RH + AM	3.68	2.02	0.55
RBW750	NRH	0.36	1.13	3.11
RBW750	NRH	0.43	0.32	0.75
RBW750	RH	1.06	0.41	0.39
RBW750	RH	1.07	0.45	0.42
RBW750	RH	0.77	0.23	0.29
RBW750	RH	1.30	0.59	0.46
RBW750	RH	3.90	1.66	0.43
RBW750	RH + AM	2.61	1.51	0.58
RBW750	RH + AM	0.93	0.33	0.35
RBW750	RH + AM	3.54	1.80	0.51
RBW750	RH + AM	2.86	1.00	0.35

Table A2.13 (Continued)

TSP	RH	2.69	0.91	0.34
TSP	RH	2.68	0.68	0.25
TSP	RH	1.32	0.47	0.36
TSP	RH	2.69	0.95	0.35
TSP	RH	1.75	0.68	0.39
TSP	RH + AM	11.31	0.52	0.05
TSP	RH + AM	5.34	2.64	0.49
TSP	RH + AM	4.64	2.59	0.56
TSP	RH + AM	3.99	1.14	0.29
TSP	RH + AM	5.91	2.50	0.42

Table A2.14: P concentration of shoot and root maize biomass receiving no additions (NP), rendered bone char produced at 750°C (RB750), co-pyrolyzed rendered bone-wood char produced at 750°C (RBW750) or triple superphosphate (TSP) fertilizer. Maize soil exploration capacity (SEC) is altered by with the absence of root hairs (NRH), root hairs (RH) and inoculation with arbuscular mycorrhizae (RH + AM).

P source	Soil exploration capacity	Shoot P concentration (mg g^{-1})	Root P concentration (mg g^{-1})
NP	NRH	1.19	1.05
NP	NRH	1.08	1.01
NP	NRH	1.29	1.06
NP	RH	0.97	1.04
NP	RH	1.20	1.07
NP	RH	1.12	0.95
NP	RH + AM	1.36	0.88
NP	RH + AM	1.29	1.02
NP	RH + AM	1.26	1.20
NP	RH + AM	1.41	0.86
NP	RH + AM	1.27	1.23
RB750	NRH	1.35	1.33
RB750	NRH	1.31	1.03
RB750	RH	1.31	0.95
RB750	RH	1.37	0.96
RB750	RH	1.26	1.19
RB750	RH	1.33	0.58
RB750	RH	1.22	0.60
RB750	RH + AM	2.47	1.47
RB750	RH + AM	1.82	1.24
RB750	RH + AM	1.41	1.51
RB750	RH + AM	2.01	1.94
RB750	RH + AM	1.77	1.36
RBW750	NRH	1.14	5.64
RBW750	NRH	1.09	0.91
RBW750	RH	1.22	0.88
RBW750	RH	1.35	0.99
RBW750	RH	1.36	0.66
RBW750	RH	1.28	0.87
RBW750	RH	1.73	1.33
RBW750	RH + AM	1.71	1.48
RBW750	RH + AM	1.83	0.88
RBW750	RH + AM	2.27	1.46
RBW750	RH + AM	2.05	1.03

Table A2.14 (Continued)

TSP	RH	1.56	0.98
TSP	RH	1.49	0.79
TSP	RH	1.54	0.86
TSP	RH	1.34	0.74
TSP	RH	1.39	0.82
TSP	RH + AM	4.13	0.36
TSP	RH + AM	1.83	1.50
TSP	RH + AM	1.36	1.26
TSP	RH + AM	1.38	0.65
TSP	RH + AM	1.80	1.16

Table A2.15: Total length proportions (%) of maize root orders. P source included no phosphorus (NP), rendered bone char produced at 750°C, rendered bone-wood char produced at 750°C and triple superphosphate (TSP) fertilizer. Soil exploration capacity of maize root system was varied by not having root hairs (NRH), having root hairs (RH), having root hairs and being inoculated with arbuscular mycorrhizae (RH + AM).

P source	Soil exploration capacity	Proportion Order 1 (%)	Proportion Order 2 (%)	Proportion Order 3 (%)
NP	NRH	73	27	0
NP	NRH	72	28	0
NP	NRH	62	38	0
NP	RH	77	23	0
NP	RH	66	34	0
NP	RH	78	22	0
NP	RH + AM	2	77	21
NP	RH + AM	1	99	0
NP	RH + AM	67	15	18
NP	RH + AM	8	79	13
RB750	NRH	61	39	0
RB750	NRH	69	31	0
RB750	RH	9	74	17
RB750	RH	5	78	18
RB750	RH	2	75	23
RB750	RH	2	82	16
RB750	RH + AM	12	80	8
RB750	RH + AM	6	83	12
RB750	RH + AM	6	87	7
RB750	RH + AM	5	83	13
RBW750	NRH	0	72	27
RBW750	NRH	78	22	0
RBW750	RH	84	16	0
RBW750	RH	0	81	19
RBW750	RH	2	83	15
RBW750	RH + AM	2	91	7
RBW750	RH + AM	69	31	0
RBW750	RH + AM	8	79	12
RBW750	RH + AM	2	79	19
TSP	RH	2	80	18
TSP	RH	1	85	15
TSP	RH	3	83	14
TSP	RH + AM	8	84	8
TSP	RH + AM	8	79	14
TSP	RH + AM	90	10	0

Table A2.16: Total surface area proportions (%) of maize root orders. P source included no phosphorus (NP), rendered bone char produced at 750°C (RB750), rendered bone-wood char produced at 750°C (RBW750) and triple super phosphate (TSP) fertilizer. Soil exploration capacity of maize root system was varied by not having root hairs (NRH), having root hairs (RH), having root hairs and being inoculated with arbuscular mycorrhizae (RH + AM).

P source	Soil exploration capacity	Proportion Order 1 (%)	Proportion Order 2 (%)	Proportion Order 3 (%)
NP	NRH	34	66	0
NP	NRH	44	56	0
NP	NRH	25	75	0
NP	RH	33	67	0
NP	RH	27	73	0
NP	RH	44	56	0
NP	RH + AM	1	44	55
NP	RH + AM	0	41	59
NP	RH + AM	50	50	0
NP	RH + AM	5	57	38
RB750	NRH	28	72	0
RB750	NRH	34	66	0
RB750	RH	4	46	50
RB750	RH	2	51	46
RB750	RH	1	43	57
RB750	RH	1	50	49
RB750	RH + AM	4	68	28
RB750	RH + AM	3	65	33
RB750	RH + AM	3	73	25
RB750	RH + AM	2	56	42
RBW750	NRH	0	100	0
RBW750	NRH	44	56	0
RBW750	RH	50	50	0
RBW750	RH	0	50	50
RBW750	RH	1	57	43
RBW750	RH + AM	1	74	25
RBW750	RH + AM	34	66	0
RBW750	RH + AM	4	63	34
RBW750	RH + AM	0	48	51
TSP	RH	1	48	51
TSP	RH	0	59	40
TSP	RH	1	55	44
TSP	RH + AM	3	66	31
TSP	RH + AM	3	57	40
TSP	RH + AM	70	30	0

Table A2.17: Morphological characteristics of maize root system including number of root orders, number of first-order root tips and specific root length (SRL). As P source, maize received no addition (NP), rendered bone char produced at 750°C (RB750), co-pyrolyzed rendered bone-wood char produced at 750°C (RBW75). Maize soil exploration capacity is varied by having no root hairs (NRH), root hairs (RH) and root hairs with AM inoculant (RH + AM).

P source	Soil exploration capacity	Number of orders	1st order tips (number 10 cm^{-1})	SRL ($m\ g^{-1}$)
NP	NRH	2	51	10.2
NP	NRH	2	40	6.2
NP	NRH	2	52	6.8
NP	NRH	2	70	7.8
NP	RH	2	37	7.5
NP	RH	2	53	9.7
NP	RH + AM	3	11	8.3
NP	RH + AM	3	4	6.6
NP	RH + AM	2	47	8.9
NP	RH + AM	3	35	10.0
RB750	NRH	2	34	7.3
RB750	NRH	2	53	8.4
RB750	RH	3	17	8.5
RB750	RH	3	18	6.3
RB750	RH	3	8	7.7
RB750	RH	3	25	7.0
RB750	RH + AM	3	85	8.7
RB750	RH + AM	3	45	9.4
RB750	RH + AM	3	67	7.6
RB750	RH + AM	3	23	6.0
RBW750	NRH	3	0	7.9
RBW750	NRH	2	51	6.9
RBW750	RH	2	61	8.5
RBW750	RH	3	6	10.5
RBW750	RH	3	6	7.9
RBW750	RH + AM	3	34	7.3
RBW750	RH + AM	2	24	5.7
RBW750	RH + AM	3	35	9.3
RBW750	RH + AM	3	12	3.6
TSP	RH	3	15	7.1
TSP	RH	3	11	7.5
TSP	RH	3	30	6.6
TSP	RH + AM	3	77	5.6
TSP	RH + AM	3	36	3.9
TSP	RH + AM	2	65	5.6

Table A2.18: First-order root characteristics of maize receiving no addition (NP),), rendered bone char 750°C (RB750), co-pyrolyzed rendered bone-wood char 750°C (RBW750) or triple superphosphate (TSP). Maize soil exploration capacity (SEC) is altered by with the absence of root hairs (NRH), root hairs (RH) and inoculation with arbuscular mycorrhizae (RH + AM).

P Source	SEC	Average root diameter (mm)	Average root length (cm)	Average root surface area (cm^2)
NP	NRH	0.25	0.44	0.03
NP	NRH	0.27	0.55	0.05
NP	NRH	0.33	0.30	0.02
NP	RH	0.21	0.26	0.01
NP	RH	0.24	0.46	0.03
NP	RH	0.29	0.46	0.04
NP	RH + AM	0.20	0.07	0.00
NP	RH + AM	0.27	0.08	0.00
NP	RH + AM	0.25	0.59	0.04
NP	RH + AM	0.19	0.15	0.01
RB750	NRH	0.32	0.39	0.03
RB750	NRH	0.26	0.34	0.02
RB750	RH	0.15	0.26	0.02
RB750	RH	0.23	0.15	0.01
RB750	RH	0.23	0.09	0.00
RB750	RH	0.25	0.05	0.00
RB750	RH + AM	0.14	0.15	0.01
RB750	RH + AM	0.23	0.10	0.01
RB750	RH + AM	0.27	0.10	0.01
RB750	RH + AM	0.20	0.14	0.01
RBW750	NRH	0.11	0.30	0.01
RBW750	NRH	0.29	0.44	0.03
RBW750	RH	0.25	0.63	0.04
RBW750	RH	0.25	0.04	0.00
RBW750	RH	0.21	0.18	0.01
RBW750	RH + AM	0.25	0.07	0.00
RBW750	RH + AM	0.32	0.62	0.05
RBW750	RH + AM	0.24	0.16	0.01
RBW750	RH + AM	0.36	0.07	0.00
TSP	RH	0.19	0.07	0.00
TSP	RH	0.34	0.04	0.00
TSP	RH	0.33	0.08	0.00
TSP	RH + AM	0.19	0.11	0.01
TSP	RH + AM	0.25	0.13	0.01
TSP	RH + AM	0.40	0.36	0.04